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UNIVERSITY OF ALBERTA

# Texture Improvement of Meat-based Food Analogs with Rhubarb Fiber

by

**Chandrani Atapattu** ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF **Master of Science**

IN

**Food Processing**

**Department of Food Science**

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Date: Feb. 22, 1993

## Abstract

Fabricated foods and food analogs such as meat imitations and surimi based products have gained increasing acceptance over the past two decades. Texturization procedures for these types of foods have been widely studied to provide "natural" sensory characteristics to the products. Among the most important sensory properties generally lacking in these products are chewability and mouthfeel. Adding "fibrous" texture to some of these products is likely to enhance their acceptability.

In this project, a surimi-type product was texturized using vegetable fiber. Surimi was prepared with mechanically deboned chicken meat (MDCM). Long fiber extracted from rhubarb stalks (*Rheum rhaponticum,* L.) were used as the fiber source in the texturization process. A procedure to extract meat proteins from MDCM was established. Processing variables involved in the production of raw and frozen chicken surimi and in the surimi gelling process were standardized based on the desired gel strength.

A fractional factorial experimental design ($2^{4-1}$) was used in the design of the experiments in the initial extraction process for MDCM. In subsequent experiments the number of washing cycles was fixed at 5 - the first with 0.5% $NaHCO_3$, the second with 0.06% salt and the rest with 0.3% salt solutions - to maximize color removal and to minimize the loss of protein in the extracted samples. The concentration of the salt solution and the mixing time with the salt solution in the washing process were set at 0.3% and 7 min, respectively. For optimum freeze-thaw stability of the extracted chicken surimi, the concentration of cryoprotectant (a mixture of sorbitol, sucrose and polyphosphate) was set at 11.4 weight percent.

A fractional factorial experiment ($2^{4-1}$), and subsequently $2^3$ full factorial experimental design were used to optimize gelling time, temperature and

fiber level, using the range values of 2-6 h, 43-55°C and 5-15%, respectively. Gelling time was found to have significant effect on texture. Gelling temperature did not significantly affect the texture within the range used in the experiment. Inclusion of 15% rhubarb fiber (w/w) showed an effect ($p=0.08$) on the texture of the end product.

A preliminary sensory analysis of the fiber-incorporated surimi gel was conducted to determine the desirable gel strength. Subsequently, the effect of rhubarb fiber on the texture of the end product was evaluated using a sensory panel. Chicken surimi gels with 20% and 0% rhubarb fiber were compared. The texture of the samples with fiber was preferred by the sensory panel. Physical properties of rhubarb fiber appeared to be responsible for the texture improvement of chicken surimi gel.

Scanning Electron Microscopy (SEM) examination of the surimi samples showed that a gel matrix was formed by salt soluble protein within the helical coils of rhubarb fiber. SDS- page analysis indicated a high concentration of myosin in samples incorporated with rhubarb fiber. Microbiological populations of samples were analyzed at critical hazardous points to ensure the safety of the product. Presence of *Salmonella* was detected in extracted meat proteins, but not in samples gelled at 55°C or deep fried. Washing reduced the total bacterial count. Gelling at 55°C for 4 h reduced the bacterial count to an undetectable level.

It was concluded based on the subjective and objective analyses of the texturized product that the addition of 20% rhubarb fiber improved the mouthfeel of chicken surimi gel produced under the standardized conditions established in this research, and in which chicken surimi was heated at 55°C for 4 h.

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# Chapter 1. Introduction and Literature Review

## 1.1. General introduction

In recent years, texture modification of meat proteins has become an important area of research in food technology. Surimi production using fish protein is a long established process which pioneered the technologies relevant to this texture modification study. Demand for novelties and value-added food products in general, including surimi-type, has increased as a result of changing life styles, health reasons, better education, etc., as well as government policies. The Japanese traditional arts of food processing bear major responsibility for the introduction of surimi-type products to the world market. Awareness of better technologies and methods as well as consumer needs have encouraged researchers to continue to explore the area of texture modification in order to improve sensory properties of these food items.

Surimi-based products (kamaboko) have gained increasing commercial importance in recent years. Expansion of the kamaboko industry led to further study of their texturization techniques in order to improve the mouthfeel and meet needs of consumers. The rapid growth of the poultry industry and the development of new flesh food-based convenience foods (e.g., chicken nuggets) suggest that additional opportunities exist for all of the meat industries (Regenstein, 1986). The concept of restructuring meat and poultry products has come into wide use in the past decade (Mandigo, 1986). In addition, meat restructuring adds value and upgrades raw material which otherwise would not be a part of a consumer product.

Restructuring of meat products offers many opportunities to the food industry. Composition, portion and shape control satisfy many consumer-driven parameters for convenience-oriented meat products. More protein sources are added to the restructured meat and poultry products as well as meat analogs, because of the

processor's motivation to increase the value of specific primal cuts, utilize lean trimmings, etc. Further processing of surimi-type products has taken place where processors are especially interested in improving texture. Texturization of protein from various sources to improve the mouthfeel of the end product has been studied by a number of researchers. As a result, spinning texturization, freeze texturization, texturization by heat extrusion and chemical modification of texture have been added to food manufacturing processes.

Parallel to the demand for improved physical properties, the health aspect of food has captured a considerable interest among consumers. Marriage between these two aspects of food is beneficial to both the consumer and the processor. Use of vegetable fiber in the texturization process enables the processor to satisfy the physical properties and health aspect of food simultaneously by improving the mouthfeel and increasing the dietary fiber level in the textured product. Provided the chosen vegetable is abundant, texture modification using vegetable fiber should be a cheaper method than other available texturization methods.

Palatability represents a great challenge for fabricated foods development (Holahan, 1975). The majority of people select their food primarily for the enjoyment they derive from eating, and only secondarily for its nutritional value (Bourne, 1975). It is expected that because of its physical nature, vegetable fiber incorporated into restructured meat products would improve their mouthfeel. The structural characteristic of bundles of long fiber strands in vegetables such as celery, asparagus and rhubarb suggests their usage as sources of vegetable fiber in fabrication technology. Further processing of celery and asparagus may not be profitable due to their existing high market demand in fresh or frozen form. The value of rhubarb as food has not yet been widely appreciated except for making wines, jams, jellies, etc. Extracted fiber bundles from rhubarb would conceivably impart a more natural

texture to restructured meat proteins. The development of new uses of rhubarb would encourage and increase the need for rhubarb cultivation, thus returning North America to an era of 'rhubarb mania' as it was in the 1960s (Foust, 1992).

Although the surimi processing technology with fish protein is long established, little is known about the processing technology in which meat or poultry, e.g. mechanically deboned chicken meat (MDCM), is used as a raw material. However, food trends suggest that the surimi industry will grow in Canada and U.S. for three reasons: consumer's concern for health and well being; the increase in price and decline in supply of traditional seafood items such as lobster, shrimp, crab and scallops; and the increasing demand for convenience foods (Ooraikul et al., 1991). Although the other methods of texturization of meat proteins are well established, very little is known about texturization using vegetable fiber. Protein texturization on its own implies protein-protein interactions, and the type and extent of protein-protein binding forces determine the structural as well as the physical and textural characteristics of the textured protein products. Using vegetable fiber in the system would allow protein-polysaccharide binding forces to contribute to the protein matrix formation in texturization.

In this project, the procedure used in producing chicken surimi was adapted from that of fish surimi through a series of experiments to standardize processing variables commonly used in the process. Gelling properties and texture modification of chicken surimi, using rhubarb fiber, were studied. The acceptability of the modified texture was then determined using a sensory panel. Physical and chemical characteristics of the chicken surimi textured with rhubarb fiber were also analyzed by objective methods.

## 1.2. Objectives

The primary objective of this research was to improve the sensory properties of washed chicken protein (chicken surimi), prepared from mechanically deboned chicken meat, with the aid of rhubarb fiber. To achieve this objective experiments were divided into three parts:

a. production of chicken surimi;

b. establishing the effects of rhubarb fiber on chicken surimi gel; and

c. texturization of chicken surimi with rhubarb fiber.

Detailed studies under these experiments included the following:

i. Standardization of the extraction process for mechanically deboned chicken meat;

ii. Standardization of the chicken surimi gelling process;

iii. Study of the effect of vegetable fiber on the texturization of chicken surimi;

iv. Study of the consumer attitude towards the chicken surimi;

v. Study of the physicochemical behavior of rhubarb fiber in texture modification;

vi. Study of the safety of the process proposed in chicken surimi production; and

vii. Determination of safe storage conditions for chicken surimi.

## 1.3. Literature Review

The following literature review covers aspects of rhubarb production and consumption, the production of mechanically deboned chicken meat, protein gelation, and surimi production.

### 1.3.1. Rhubarb

The medicinal properties of rhubarb have been known for a long time as explained by Foust (1992). He quotes from Towers (1836), "Anything more productive, salubrious, profitable, and expressly suitable to the purposes of the cottager [than rhubarb], can scarcely be found in the entire list of vegetable production". Rhubarb was consumed as food prior to the nineteenth century but its use was never extensive nor widespread. Tarts and pies were the most popular items made from rhubarb. As reported by Nicholson et al. (1973) the rhubarb plant, which was already exploited in Asia as a food plant, was brought into the gardens of Europe from eastern Asia by Vasco Da Gama in 1497. According to Nicholson et al. (1973), the long fleshy leaf-stalks were usually red (green stalks were less popular).

Rhubarb farming in North America began in forcing houses, cold-cellars, and hothouses. The bulk of the crop came from the Midwest United States, i.e. from Michigan, Indiana, and Illinois, where the forcing industry had grown rapidly since 1900, particularly since the end of World War I. Forced rhubarb, which is grown early in the season, is generally preferred as it is tender and less acid than the later, unforced stalks. For forcing, the rootstocks are lifted and left on top of the soil for about two weeks, exposed to the winter weather. They are then placed in the staging or covered with barrels. The young leaf stalks develop rapidly and are usually an attractive pale red, with yellowish instead of green-leaf blades (Nicholson et al., 1973).

By the 1930s, the apogee of the fresh rhubarb industry, and prior to the spread of quick-freezing technology, the forcing houses were concentrated in Michigan's Macomb County and in the region just north of Detroit, particularly around the small towns of Warren and Utica (the latter once claimed the title "The rhubarb capital of the world") and south of Detroit around New Boston and Wayne. In the late 1950s Michigan had about 260 growers utilizing 450 forcing-buildings and 1,100 to 1,200 field acres for forcing (Foust, 1992).

Other major sources of rhubarb production were the state of Washington which had 120 growers utilizing 300 buildings and 800 acres, and Ontario which had nearly 40 growers operating 80 houses and a total of about 130 acres. The state of Washington now produces nearly 60 percent of the United States' crop (which is slightly less than 20 million pounds), and with the addition of Oregon and California, the West Coast accounts for more than three quarters of commercial rhubarb grown in the United States. The twenty six members of the Washington Rhubarb Growers' Association produced the bulk of the more than 9 million pounds grown in the State in 1988. In recent years, approximately 1.25 million pounds annually has been hothouse rhubarb, all of which is being sold as fresh crop. About two thirds of the rhubarb production are frozen by processing companies for use in the making of pies, jellies, jams, etc. All the field rhubarb (somewhat more than 500 acres) comes from the cultivar Crimson, which is red throughout and virtually virus free. The forced plants are Wine and Johnson Red, the latter being a North American selection developed about a decade ago. It grows dark red when forced, but like Victoria, is green in the field.

Marketing of Washington rhubarb, both forced and field, is now nationwide, with the eastern cities of Boston, New York, and Montreal accounting for the largest usage. Horticultural research on rhubarb continues at a modest level. The

Clarksville (Michigan) Horticultural Experiment Station, a branch of Michigan State University, claims what may be "the world's largest accumulation of rhubarb varieties at a single location". Some sixty varieties have been planted since the first one was planted in 1979, in an effort to evaluate desirable characteristics such as height, yield, sugar content, acidity, and color.

The chemical composition of rhubarb creates certain drawbacks in its use due to its high content of oxalic acid. Oxalic acid is concentrated mainly in the leaf petal but not in the stalks. Therefore, the use of rhubarb fiber in foods is not very much affected by its chemical composition (Foust, 1992). Rhubarb stalk is rich in fiber. Similar to other vegetable fibers, this fiber can be divided into two main components; soluble fiber and insoluble fiber. The long strands of rhubarb fiber are made of bundles of helical coils and provide mechanical strength to the stalk and also play a role in the internal transport of water, nutrients and metabolites.

Insoluble dietary fiber is plant material that is neither digestible by appropriately chosen enzymes that mimic the human alimentary system nor soluble in hot water. Soluble dietary fiber is food material that is not digestible by appropriately chosen enzymes but is soluble in warm or hot water. Segerlind and Herner (1972), Herner (1973), and Prosky (1992), have grouped cellulose, hemicellulose, lignin, cutin and plant waxes into insoluble dietary fiber. The main feature of the cell walls of all green plants is a framework of organized aggregates of cellulose molecules, referred to as microfibrils, embedded in a matrix of noncellulosic polysaccharides and lignin.

Cellulose is the least soluble of all fiber components. Cellulose is composed of long chains of β-1,4 linked D-glucopyranose residues containing the repeating units of cellobiose. The β-1,4 linkage allows cellulose polymer to crystallize in a linear configuration with a high degree of intermolecular hydrogen

bonding, which gives it substantial shear and tensile strength. Hemicellulose distinguishes it from cellulose due to solubility in dilute alkali. Lignin is a complex polymer of phenolpropanoid ($C_6$-$C_3$) units encrusting and penetrating the cell walls of tracheid vessels, fibers, and sclereids of vascular plants. Lignin provides mechanical strength for the plant cell walls and contributes to the texture of a variety of plant foods. Lignin tissue plays a role in the internal transport of water, nutrients and metabolites by decreasing the permeation of water across the cell walls in the conducting xylem tissues (Segerlind and Herner, 1972; Herner 1973).

In food product development, the low caloric bioavailability, ability to hold water and to interact with protein, fat and available carbohydrate make fiber a candidate for use in weight control calorie diluted products (Prosky, 1992). The structural qualities of vegetable fiber that can be useful in product development have not been reported. Research on the clinical aspect of rhubarb fiber showed that cholesterol level in mice fed with 5% dry rhubarb fiber was reduced (Basu et al., 1992). It is suggested by Foust and Marshal (1991) that renewed popularity of rhubarb is contemplated considering the development of technologies such as disease control, variation of cultivars, well established forcing technology, advanced processing and marketing techniques, and also plentiful consumer uses, compared to 100 years ago.

In Western cultures in particular, the decrease in the intake of dietary fiber appears to have resulted in an increase in appendicitis, colitis, colon cancer, constipation, coronary heart disease, Crohn's disease, diverticulitis, hyperlipidemia, ileitis, irritable bowel syndrome, maturity-onset diabetes, obesity, and varicose veins (Prosky, 1992). Addition of rhubarb fiber to food analogs, therefore, not only would improve their sensory properties, but also their health and nutritional value.

**1.3.2. Mechanically Deboned Poultry Meat (MDPM)**

Mechanically deboned poultry meat (MDPM) is a comminuted product commonly obtained from turkey frames, poultry backs and necks, or the entire carcass of spent fowl which have been processed through a mechanical deboner. The first mechanical deboning machine was developed in the late 1940's for fish processing. With the increased demand for special cuts of poultry and red meat, mechanical deboning of these materials has grown rapidly. In 1979 more than 200 million pounds of MDPM was produced in the United States (Expert Panel Report, 1979).

In mechanical deboning, the separate carcass parts, or coarsely crushed bones are forced against the screened or slotted surface of the deboner. The muscle fibers and the other edible tissue pass through the openings, while the bone particles, except for very small fragments, are retained. Deboned red meat and poultry emerges from the machine as a finely ground, paste-like product. The average size of bone fragments in mechanically deboned products ranges from 77 to 112 μ (Field et al., 1977) and the particles are completely dissolved by acid of the same strength as that found in human gastric juice. Kolbey et al. (1977) concluded that bone fragments in mechanically deboned poultry and fish do not constitute a hazard to consumers.

Composition regulations issued by the USDA on mechanically deboned red meats include: 1) fat content shall not exceed 30%; 2) protein content shall not be less than 14%; 3) protein quality shall not be less than a PER (protein efficiency ratio) of 2.5; and 4) residual calcium content from bone shall not exceed 0.75%. These specifications are met by commercial products (Field et al., 1977; Kolbye et al., 1977). Collagen protein from the skin and tendon, which is of low nutritional quality, is largely removed during mechanical deboning (Satterlee et al., 1971; Field and Riley, 1974) leaving only 2 to 4% in the mechanically processed

product. Similar regulations are expected for deboned poultry meats (Expert Panel Report, 1979).

A unique characteristic of MDPM is the relatively high level of hemoglobin which results from the release of bone marrow exposed during the deboning process. Froning (1976) found the hemoglobin content was three times higher in the meat from mechanically deboned whole fowl carcass than from its hand deboned counterpart, and that even higher levels resulted from deboning broiler back meat. The paste-like texture of MDPM limits the manner in which it can be used. Late-model deboners are capable of producing meat with a texture approaching that of ordinary ground meat, but even finer in particle size.

### 1.3.3. Meat gelation and Product texture

The most important structural functions of proteins in processed meat systems and final meat products appear to be the gel-forming, water- and fat-binding abilities. One of the main physicochemical problems arising in the manufacture of meat products is related to the elaboration of methods for controlling protein functional properties so as to obtain gels of a desirable composition, structure, rheological and other physicochemical properties. These gel properties determine, in turn, the functional, textural, as well as other sensory characteristics and nutritive value of finished meat products. The required functional properties of a protein are dictated by the desired properties of a final meat product.

Many aspects of quality of product for the consumer have to be considered throughout the processing chain including fat/lean ratio, color, the water holding capacity and perhaps most important of all, texture. Protein and other food functional additives for texture control of meat systems, are components used in developing methods for processing vegetable protein into meat-like foods (namely

meat analogs and meat extenders) or in product formulation with underutilized meat sources. Therefore, it is essential to investigate the fundamental processes of structure formation as well as mechanical and other physicochemical properties of these components. The major contributions to the formation of structures and properties of foods come from two types of food macromolecules: proteins and polysaccharides (Tolstoguzov, 1991).

There are three levels of understanding of the texture in a food product. As described by Lillford (1992), textural parameters and popular nomenclature for meat-like foods are: 1) primary characteristics eg., hardness, cohesiveness, viscosity, elasticity, adhesiveness, 2) secondary parameters eg., brittleness, chewiness, gumminess 3) popular terms eg., soft→ firm→ hard, crumble→ crunch→ brittle, tender→ chewy→ tough, short→ meal→ paste→ gummy, thin→ viscous, plastic→ elastic, sticky→ tacky→ gooey. It has become a challenge to food product designers to achieve these specific parameters in their products.

Texture in soft manufactured foods based on fish proteins has been studied in detail by Rodger (1992). According to his study, about 80% of the muscle tissue volume is occupied by the myofibrils, which are filamentous in nature (~ 1μm in diameter) and run the length of the cell. The proteins which comprise the major part of this system are myosin and actin, two sets of repeating filaments (thick and thin) which are aligned parallel to the long axis of the myofibril. Application of heat to fish has a major effect on the physicochemical properties of this system. The influence of heat on the expulsion of water and tissue shrinkage, occurs as the tissue is heated. The temperature rises with little observable effect until the thermal denaturation temperature of myosin (about 37°C for most fish) is reached. The myofibrils begin to shrink as a result, causing 1) a spontaneous expulsion of

myofibrillar water into the extracellular space, 2) an effective concentration of the myofibrillar protein, and 3) an increase in the fiber rigidity. As the temperature continues to rise, the collagen in the connective tissue also begins to undergo thermal denaturation and shrinkage which compounds the expulsion of water from the cell, thus increasing protein concentration. Finally, at about 65°C, the actin component also denatures. Rodger (1992) determined this sequence of events using differential scanning calorimetry. As far as the author is aware, connective tissue contributes very little, if anything, to the sensory texture of thermally processed fish.

The response of myofibrillar proteins to acid and salt has been explained (Rodger, 1992) by referring to their isoelectric point. If the pH is shifted in any direction away from the isoelectric point (IEP) then the proteins attain either a net negative or a net positive charge resulting in increased electrostatic repulsion of the proteins. Since the pH of fish flesh post rigor (6.2-6.8) is greater than the IEP, the progressive addition of acid to pH 4 should see the structure shrink as the pH approaches the IEP (~5.4) and then begin to swell. However, if salt is present, then the pH at which the water binding of the system is minimal is shifted to a lower pH value (~4), which is much nearer the equilibriated pH of the acid-salt treatment.

To generate texture in surimi-type products two main events must occur: i.e., the creation of a protein gel and the superimposition (directional orientation) of the structure. The addition of salt to the formulation ensures a degree of solubilisation of the myofibrillar proteins, which then partially gel during the first heating stage (when two stages of heating are practiced) in the process. Slicing this gel with a strip cutter introduces 'fracture planes' along the long axis of the product. The slices are then laid one upon another. The second heating stage completes the thermal denaturation of the myofibrillar proteins and creates a stable structure. (Rodger, 1992). No evidence is currently available as to what influence varying the

dimensions of the strips has on the resultant sensory quality, but, according to Lillford (1992), the dimensions of fibers created in a process should match those of the tissue food being stimulated.

### 1.3.4. Surimi

Surimi is a product made of mechanically deboned fish flesh, mixed with cryoprotectants to improve its oxidative stability and hence its frozen shelf life. It is used as an intermediate product for a variety of fabricated seafoods (Lee, 1984). Much research has been devoted to the development of new surimi-based products in recent years (Hsu, 1990). Two types of surimi are currently manufactured, namely, raw surimi and frozen surimi. As described by Enriquez et al. (1990) raw surimi is minced meat, washed to remove fat and water-soluble constituents. When raw surimi is mixed with anti-denaturants and frozen, the product is called frozen surimi.

Various conditions and procedures have been established in surimi preparation by numerous researchers. However, basically it is a process of repeated washing of mechanically separated fish flesh with chilled water to remove colors, odors and fat. The surimi processing procedures using different types of fish species have been improved through the years. It has been suggested that the procedure can be modified to suit any new type of raw material used in surimi processing

Lee (1984) has recommended at least 3 washing cycles with chilled water; with the last washing, a 0.01% - 0.3% NaCl solution is used to ease the removal of water. In a commercial process, the washing is continuous with mechanical agitation in a series of washing tanks and rotary screen rinsers. During repeated washing with continuous agitation, much of the water-soluble protein is removed, along with undesirable substances and enzymes, causing the level of actomyosin to increase. The level of functional actomyosin is a measure of the gel-

forming ability of the surimi. Lee (1984) explains that this is why surimi gives a more elastic texture than unwashed minced fish meat. In early studies it was suggested that 5-10 washing cycles with water (v/w) be used. In another report Lee (1986), recommends that 3:1 and 4:1 water to meat ratio is adequate as well as economical in washing. He also suggests that, 0.1-0.2% salinity of water in the last washing aids the dewatering process.

During washing the temperature should be maintained between 5-10°C. Although the water temperature does not need to be kept unnecessarily low it may vary according to the type of fish (species), specifically the thermostability of the fish protein. In a later study Lee and Kim (1987) used only 2 washing cycles at 10°C, using 1 part fish meat to 4 parts water. Each of the washing cycles of 10 min was followed by draining in a rotary rinser. Ablett et al. (1991) in their study used 3 cycles of washing with 1:3 mince to water ratio. In their process, 0.06% (w/v) NaCl was added to the initial washing cycle, as previous studies had established improved gel-formation with lean gadoid species (any marine fish of the cod family Gadidae, including haddock and whiting) following inclusion of salt at this level. The subsequent washing cycles were carried out with 0.3% (w/v) salt solutions in this study.

When surimi is manufactured using dark fleshed fish (Enriquez et al., 1990), 0.5% sodium bicarbonate solution has been used in the first washing cycle and then followed by washing with chilled water and a further 2 washing cycles with 0.3% salt solution. The ratio of water to mince was varied from 2:1 to 5:1 (w/v) (Enriquez et al., 1990). Each washing cycle was followed by a dewatering process. Hsu (1990) has used a hydraulic press to dewater the washed fish mince. Lee (1986) and Lee and Kim (1987) used screw presses for dewatering. Ablett et al. (1991) dewatered in a basket centrifuge, as did Castaigne et al. (1990).

In surimi processing with fish mince, the dewatering meat slurry was strained to remove bone pieces, scales and dark skins (Lee, 1986 and Lee and Kim, 1987; Castaigne et al., 1990; Enriquez et al., 1990; and Ablett et al., 1991). In process improvement research carried out by Sierra et al. (1991) to obtain surimi from the underutilized parts of hake, the variables considered were: number of washings, water:minced fish paste ratio, washing time and percentage of NaCl in the washing solution. They examined the number of washings, 1, 2, 3 and 4; water: fish paste ratio, 2:1, 3:1, 4:1 and 5:1; percentage of salt in the water, 0.1, 0.2, 0.3 and 0.4; and the time of washing, 5, 10, 15 and 20 min. They concluded that the optimum processing steps are: number of washes 3; water:paste ratio, 4:1; time of washing, 10 min; salinity of water solution, 0.2%. Dewatering was carried out in two steps, first by a manual straining and then centrifuging in a basket centrifuge. Canto et al. (1991) has used water:fish paste ratio 5:1 with a water temperature 5-7°C. He used 0.5% $NaHCO_3$ in the first wash in order to increase the pH of the slurry.

Cryoprotectants are added to raw surimi to protect protein denaturation in freezing (Lanier and MacDonald, 1991). Lee (1986) suggested a cryoprotectant mix of sugar, sorbitol and polyphosphates mixed into dewatered flesh at levels of 4, 4, and 0.2% respectively. The same composition of cryoprotectants were used by Hsu (1990), Ablett et al. (1991) and Sierra et al. (1991). Researchers have paid more attention to this area of surimi processing in order to improve the gel forming ability of the end product. Canto et al. (1991) in his study of protein denaturation in frozen storage compared monosodium glutamate (3%), lactose (3%), milk whey (3.85%) and a mixture of glucose (4%), sorbitol (4%) and polyphosphate (0.2%) as cryoprotectants. According to Canto et al. (1991) monosodium glutamate was one of the best choices. The commercial mixture (4% glucose, 4% sorbitol, 0.2% polyphosphates) gave the best water retention quality. Whey produced minimum solubility and maximum enzymatic activity. Castaigne et al. (1990) compared the

effects of cryoprotectants on the storage stability in 14 different treatments and found the greatest stabilizing effect to cod-surimi proteins was obtained from carbohydrate/polyol treatment and sucrose/sorbitol at 8% w/w (mixture of 1:1) level.

There are differences of opinion as to the type of mixer best used in incorporating cryoprotectants; a silent cutter-type mixer or the ribbon blender-type mixer without a knife cutter. Lee (1986) reported that the silent cutter type allows better dispersion of cryoprotectants than the blender type mixer.

Surimi gel formation by temperature-time treatment has been studied by a number of researchers. These temperature-time combinations vary over a wide range. Wilson et al. (1990) compared the gel strengths of unwashed and washed surimi samples by cooking at 60°C for 40 min and 90°C for 40 min. Lee (1984) reported that surimi paste gels rapidly upon heating at 80-90°C but slowly at 40-50°C. Cooking a gel which has been slowly set at 40-50°C results in a stronger gel than cooking without a slow set. A gel network is formed by cross-linking of actomyosin with the aid of both hydrogen and hydrophobic bonds, and water is retained within the network. Hydrogen bonding is primarily involved in gel setting at low temperature, while hydrophobic bonding dominates in subsequent gel setting at high temperature. Elasticity and resilience of surimi gel increase with an increase in the concentration of actomyosin, but decrease with an increase in the concentration of water-soluble protein. The presence of water-soluble protein retards gel setting by interfering with the actomyosin cross-linking process.

Most of the surimi based products are prepared in the cooked form and stored refrigerated or frozen (Hultin et al., 1984; Lee, 1986). Some molded products are frozen uncooked. Ablett et al. (1991) prepared surimi gel by inclusion of 3% NaCl (w/w) in the thawed surimi paste. The protein sol was subsequently de-aerated in a vacuum packaging machine. Hujita and Makinodan (1990) in preparation of

kamaboko (surimi gel) ground the thawed surimi for 30 min. with the addition of 2.5% of the weight of the meat of NaCl. In his study he conducted SDS polyacrylamide gel electrophoresis to determine the texture degradation of surimi gel that contained uncooked "Judas' ear mushroom". He concluded that the degradation was caused by the activity of proteinase enzyme of this mushroom, on fish proteins. Hujita and Makinodan (1990) concluded that exudates from the mushroom hydrolyze the fish meat protein, causing textural degradation of kamaboko.

The effect of processing on yield and composition of spent hen was studied by Babji and Kee (1991). The research was limited to meat extraction and proximate analyses. He concluded that chicken surimi can be successfully produced from spent hen meat.

Actomyosin is the most important component in meat protein, where manufacture of surimi-type food items are concerned. This is because it manipulates the gel strength or texture of these products. The functional properties of these proteins can be analyzed both subjectively and objectively. Processing of MDCM in surimi manufacture has to be aimed at improving this gelling property by adjusting the processing variables involved.

Previous research on fish surimi manufacture suggests that the important variables affecting the gel strength of the end product include number of washes, the ratio of mince to washing water, concentration of salt solutions used in washing, temperature-time combinations in gelling, and the amount of cryoprotectant used. Determination of the effects of these variables, where MDCM is used as the raw material, promises to be an appropriate way of standardizing the texture of chicken surimi gel.

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# Chapter 2. Chicken Surimi Production

## 2.1. Introduction

In surimi production, fish protein is extracted with water to remove strong flavors, pigments and nonfunctional proteins, and is subsequently dewatered to reduce the moisture content to approximately that of intact fish muscle, (approximately 76-79%). The washing process converts an otherwise highly colored and often strong flavored fish mince into a highly functional, bland and light-colored fish muscle protein which has remarkable binding and texturizing properties in fabricated foods (Hamann, 1988).

The raw material used in this project, MDCM, is high in fat content, approximately 20% (Ooraikul et al., 1991). It also contains bone marrow which is high in hemoglobin. This increases the color pigment level in the raw material (Expert Panel Report, 1979). In meat protein extraction process, it is necessary to remove these undesirable components, i.e. fat and color pigments, while improving the gel forming ability of the extract.

Processing variables involved in the protein extraction process from fish mince were studied by Sierra et al. (1991). His studies covered the effects of such variables as number of washes, concentration of salt solution used in washing, water:mince ratio and mixing time, on the gel strength of the surimi.

Raw surimi which is a concentrated product of myofibrillar proteins, suffers physical damage as a consequence of freezing and cold storage. The use of cryoprotectants to prevent or minimize this damage in frozen storage, in order to preserve gel forming ability of the surimi, has been studied by a number of researchers (Park and Lanier, 1987; Green et al., 1988; Castaigne et al., 1990; Canto

et al., 1991; Lanier and MacDonald, 1991, and Lee et al., 1991). Castaigne et al. (1990) investigated salt extractable proteins (SEP) in cod samples stored with different cryoprotectants at -12°C for a period of 16 weeks. This study was carried out to compare the effectiveness of different cryoprotectants on the stabilizing effect of cod-surimi proteins. They reported that the greatest stabilizing effect on cod-surimi protein was obtained when carbohydrate/polyol at the 8% w/w level was used. They also suggested that further research is needed on carbohydrate/polyols to improve understanding of the mechanisms of the fish protein stabilization.

In the present study, the effect of similar processing variables, such as number of washes, concentration of the salt solution, mixing time and the amount of cryoprotectant, on the texture of chicken surimi was investigated. Results of this experiment were used in optimization experiments, in optimizing the number of washes to be used in the meat protein extraction process and in obtaining an optimum texture level for the chicken surimi gel through a sensory evaluation.

## 2.2. Materials and Methods

The source of meat protein used in this research project was mechanically deboned chicken meat (MDCM). It was chosen because of its availability and low cost as compared to other deboned meats. Other ingredients used in the process were salt and cryoprotectants which consisted of sorbitol, sucrose and potassium polyphosphate.

### 2.2.1. Mechanically Deboned Chicken Meat (MDCM)

Mechanically deboned chicken meat was obtained from Lilydale Poultry Processing Company, Edmonton, Alberta. This MDCM was produced from used hens, back portions, and chicken necks. The deboned meat was immediately chilled in a chilling machine and then frozen. Samples obtained for this research were

extruded from mechanical deboning machine in 5 kg portions into polyethylene bags and frozen in waxed cardboard boxes. The boxes were stored at -30°C until used.

MDCM contains chicken skins, fat, meat protein, water, color pigments and minerals extracted from the original raw materials. MDCM is, therefore, a minced meat, which is non-homogeneous, and pink in color, and the particle size can vary quite widely. Preliminary studies were carried out on protein extraction from MDCM in order to identify and standardize the processing variables involved in the process. Important variables were then selected to be used in subsequent experimental designs. Precautions were taken in the processing of the highly perishable MDCM to ensure safety and to preserve the functional properties of the end product. Ten kilograms of meat were processed in each trial. The procedure followed in the protein extraction from MDCM is shown in Figure 2.1.

**2.2.1.1. Thawing and Crushing**

Boxes containing frozen mechanically deboned chicken samples were left in the cold room (4°C) overnight. Further 1-2 h thawing was allowed at room temperature (15-20°C) prior to processing. Thawed MDCM blocks were cut into approximately 4x4x2 $in^3$ (~10x10x6 $cm^3$) chunks. Subsequently, the chunks were broken into finer pieces in a silent cutter.

**2.2.1.2. Washing to remove fat and color pigments**

Removal of fat and color pigments was carried out in a series of washings using a Hobart mixer. The material was mixed at a low speed setting, using a 'K' beater. Due to low density, fat separated from the meat proteins by floatation while the protein settled to the bottom of the vessel. Ten-kg batches of chopped MDCM were mixed with salt solutions at a ratio of 1:4 in each series following the quantities used by Ablett et al. (1991). In their study of minced fish, Ablett et al

(1991) washed the fish with water at a ratio of 1:3. However, due to the high contents of fat and color pigments in MDCM, a greater volume of washing solution was used in this project. Following the procedure used by Enriquez et al. (1990), the first wash was carried out with 0.5% $NaHCO_3$ to maintain a neutral pH during the leaching process, which enhances the product gel strength (Enriquez et al., 1990) and may have some microbiological inhibition value. Dipping cod fillets in sodium bicarbonate solution markedly reduced microbial growth (Montville et al., 1990). After the first wash, the bottom portion of the mix which contained meat proteins and wash solution was siphoned out through a plastic tube (diameter 2.5 cm). The remaining fat layer was further washed with 0.5% $NaHCO_3$ solution and the extracted meat proteins from the fat layer were added to the rest of the meat protein extract. Fat was discarded and meat protein extract was subjected to further washing. The second wash of the extracted proteins was carried out with 0.06% NaCl solution followed by 3 washing cycles with 0.3% NaCl solution. This follows the Japan Surimi Association (JSA) recommended method which involves three leaching cycles for dark-flesh fish mince. After preliminary protein extraction experiments with MDCM, it was decided that 5 washes were necessary for the extraction. First wash: in 0.5% $NaHCO_3$ solution; amount, four times the weight of extract; duration, 10 min. Second wash: in 0.06% NaCl solution; amount, four times the weight of extract; duration, 7 min. Third, fourth and fifth wash: in 0.3% NaCl solution; amounts, four times the weight of extract; duration, 7 min each. The solutions were maintained at temperatures not lower than 6°C or higher than 10°C. This has been reported to be the ideal temperature range for separation of fat from the meat proteins (Enriquez et al., 1990; Canto et al., 1991; Ablett et al., 1991).

**2.2.1.3. Dewatering**

Each washing cycle was followed by a dewatering process. The most common dewatering methods in surimi processing are centrifugation in a basket centrifuge or screw pressing (Lee, 1984; Enriquez et al., 1990). However, both these methods were found to be impractical in this project. When the basket centrifuge was used after initial washing cycles, the holes on the basket were blocked due to the high fat content of the MDCM, even after removing the fat layer floated on the top of the washed solution. It was time consuming, and also could cause undesirable changes in muscle protein structure and an increase in microbial population. Dewatering in the screw press was efficient, but it destroyed the protein matrix which did not regain its original state (as observed in scanning electron microscope) in further washing.

In this study dewatering was done through a nylon mesh bag (mesh size: 0.5 x 0.5 $mm^2$) after each of the washing cycles. After the final wash, washed water was strained through the nylon mesh bag and remaining meat protein extracts were further dewatered in a basket centrifuge ('International Centrifuge' Model S2K, International Equipment Co., Needham HTS., Mass., U.S.A). The wall of the basket was lined with a coarse filter paper (Whatman 1454-917, 57L x 46W (cm) sheets) to prevent loss of solid particles with drained water.

**2.2.2. Other ingredients**

Salt (NaCl) manufactured by Sifto Canada Inc., Mississauga, ON was used in preparing salt solutions for washing in the production of chicken surimi from the MDCM, and in adding to the raw chicken surimi to aid the gelling process. Cryoprotectant used in the project was prepared by mixing 4 parts of food grade sorbitol, a 70% aqueous solution (Pfizer Scientific Company, Toronto, ON), and 4 parts of sucrose (extra fine granulated Berry sugar, Rogers' Sugar Refining Company

Ltd., Vancouver), with 0.2 parts of potassium polyphosphate supplied by Vans' Sausage Co., Edmonton, Alberta. Sodium bicarbonate (USP grade) used in the first cycle of washing was a product of Church Dweight Ltd., Don Mills, ON.

#### 2.2.2.1. Cryoprotectant mix

Cryoprotectant mix prepared as described above was a clear, colorless, viscous solution. Sorbitol used was a 70% aqueous solution. Therefore, the adjusted ratio used in this experiment was 5.71g sorbitol aqueous solution : 4g sucrose : 0.2g potassium polyphosphate. A large batch of mixture (approximately 1L) was prepared and used throughout the project to prevent variation due to weighing and mixing. Polyphosphates and sucrose were dissolved in 60g water at 60°C. Sorbitol was mixed into the solution with the aid of a magnetic stirrer. The mixture was dispensed into 90 mL Pyrex bottles and heated at 80°C for 2 h to prevent fungal growth during the storage. The bottles were stored at refrigerated temperature (4°C) until further use.

### 2.2.3. Experimental design in meat protein extraction

A one-half fractional factorial design ($2^{4-1}$), i.e. a balanced design, was used following the procedure described by Box et al. (1978). Processing variables and their low and high levels considered in the meat protein extraction experiments are shown in Table 2.1. Eight experimental units were taken for the $2^{4-1}$ experimental design. Each unit was coded with a three digit random number. The sequence of the units was randomized to reduce systematic error.

Processing variables included in this experiment at the initial stage were: number of washing cycles, concentration of the salt solution used in washing, mixing time of meat with salt solution, and the level of cryoprotectant added as a percentage of the weight of extracted meat. The effect of rhubarb fiber on product texture was to be determined in subsequent studies.

Meat protein extraction was carried out following the procedure described in Figure 2.1. Two boxes of meat, 5 kg each, were thawed at 4°C overnight and then at room temperature (15-20°C) for 2 h. Partially thawed meat blocks were cut into chunks of approximately 10 x 10 x 5 $cm^3$ and then crushed into finer pieces in the silent cutter.

Partially thawed MDCM, finely crushed in the silent cutter, were washed with salt solutions in the Hobart mixer (Hobart Manufacturing Co. Ltd., Don Mills, ON), at the lowest speed. Salt solution and MDCM were mixed at a ratio of 4:1 in the washing process. The liquid phase which contained meat proteins was siphoned out and the fat layer remaining in the mixing bowl was discarded. This mixture was strained through a nylon mesh bag (mesh size 0.5 x 0.5 $mm^2$) and the washed water which contained water soluble material was discarded. Extracted meat proteins were further washed with salt solutions at a ratio of 1:4. The concentration of salt solution used in washing mixing time with the salt solution, and the number of washes used were determined according to the experimental unit. The temperature of all the solutions used in washing, was maintained between 6-10°C. After each of the washes meat proteins were dewatered by straining through the nylon mesh bag. After the final wash, meat protein extracts were further dewatered in a basket centrifuge (International Centrifuge, Model S2K), lined with coarse filter paper (Whatman 1454-917, 57L x 46W (cm) sheets). Cryoprotectant mixture was mixed into the extracted meat at percentages indicated by the experimental design. Extracted meat samples were further processed following the procedure shown in Figure 2.2.

Color was analyzed for whiteness (L) and redness (a) using a Hunter Color meter calibrated with the standard cream color plate, a color close to that of the processed meat. Samples were vacuum packed in a cryovac Nylon-Polyester bag (UNIWEST, Edmonton, Alberta) using a Multivac vacuum packaging equipment

(Type AG-500, Made in W. Germany) and stored at -30°C overnight. The frozen samples were then thawed at room temperature for a period of 3 h and mixed with 3.0% dry salt (Sifto) in a silent cutter (Model 84185, Hobart Manufacturing Co. Ltd., Don Mills, ON). "Sausages" of equal size were made from the mixture using polyethylene casing and cooked in a water bath at 90°C for 30 min. The polyethylene casings were removed after samples had reached the room temperature. Product texture was analyzed as described below (Section 2.5.1).

The $2^{4-1}$ fractional factorials were analyzed using analyses of variance. Sources of identified variations were computed using Statistical Analysis System (1985). Standard errors of the mean of each product attribute were computed using the pooled estimate of error variation.

#### 2.2.3.1. Product analysis

Sausages, with polyethylene casings removed, were cut into lengths of 2.5 cm and texture was analyzed immediately after. Texture of the sausages was analyzed in the Instron texture measuring equipment with Kramer shear cell (Table model 1130) under 50 kg gauge. When the samples were cut through the outer surface (through the skin) double peaks were observed on the strip chart (Figure 2.3). The first peak which was given at the time of cutting through the surface (through the skin) was reported as 'brittleness'. The second peak representing the force required to cut through the samples was reported as 'hardness' (Bourne, 1975).

### 2.2.4. Optimization Experiments

The objectives of the studies carried out at this stage were: 1) to obtain a value for the desired texture level by subjective analysis and 2) to standardize the number of washes considering the color and yield as the dependent variables.

The desired texture level of the product was studied using a sensory panel. Results from the meat extraction experiment were used in selecting the samples to be presented for sensory analysis.

The meat protein extraction experiment showed that the effects of number of washings, salt concentration in wash-water, mixing time and the concentration of cryoprotectant on the texture of the protein extract (chicken surimi) were not significant. However, its color was significantly affected by the number of washes, concentration of salt solution and amount of cryoprotectant. Therefore, it was possible to standardize the processing variables based on yield and color of protein extract and the amount of waste water generated rather than on the texture of the extract. Although the amount of cryoprotectant showed significant effect on the color of chicken surimi, its main function is on the freeze-thaw stability of the product. Therefore, it was not included as a variable in the process optimization study. Only number of washes and concentration of salt solution were included in the study.

A number of researchers have studied the optimum number of washes when fish mince was used as the raw material in surimi processing (Ablett et al., 1991; Sierra et al., 1991; Hsu, 1990). However, no such studies have been conducted on the optimum number of washes for meat protein extraction from MDCM. Generally, excess washing results in a low yield of protein extract. However, insufficient washing leaves excess colored pigments in the end product which leads to product quality deterioration in the storage. Therefore, in the present experiment to maximize the yield of the chicken surimi with light color, the number of washes were optimized through an experiment using a series of washing cycle as indicated in the experimental design (Table 2.1).

**2.2.4.1. Optimum texture characteristics**

Two samples, one having soft and the other having hard texture, were produced and analyzed by a sensory panel to determine the type of texture preferred. The processing conditions used to produce soft and hard products were chosen from the previous experiments based on whether they produced softer or harder products even though the effects of those variables were not considered statistically significant. The processing conditions used for the softer texture were: number of washes, 3; concentration of NaCl solution, 0.5%; mixing time, 5 min; amount of cryoprotectant, 12.8%. The processing conditions for the harder texture were: number of washes, 3; concentration of the NaCl solution, 0.5%; mixing time, 15 min; and the amount of cryoprotectant, 9.98%. Because rhubarb fiber would be used as a variable in the study of gelling and texturization of chicken surimi (Chapter 3 and 4), it was decided that it should be incorporated into the surimi at this stage so that the panelists could detect the presence of the fiber in the product. However, the concentration of the fiber was kept constant at 10% as the effect of its concentration was not studied at this point.

Eleven panelists were presented with coded samples having two different textures, and a questionnaire (Figure 2.4). Panelists were asked to indicate an ideal level of texture on three horizontal lines according to their perceptions for smoothness, between "Too tough' and 'Too smooth', firmness between "Too soft' and 'Too hard' and moistness between 'Too dry' and 'Too moist', by cutting the samples with a knife and then feeling them between fingers. Overall acceptability of the product was evaluated between 'extremely unacceptable' and 'extremely acceptable'. These questionnaires were analyzed and the values given for coded samples and 'Ideal Points' were converted into a nine point scale. Means for each of the attributes, given for softer and harder samples, and 'Ideal Points' were calculated.

### 2.2.4.2. Waste water analysis

Mechanically deboned chicken samples were washed to remove fat and color pigments. The washing process was carried out in a Kenwood Chef food mixer. 'K' beater (mixing device for liquid type products) was used in mixing operation. Two different concentrations of NaCl solutions, 0.15% and 0.3% were used, one for each set of washing experiments. Washed water was analyzed for protein content, color and total solids. The meat was washed eight times. Waste water samples were analyzed after each wash for red color using Hunter color meter, calibrated with a standard pink color plate. The protein content of the waste water was analyzed using the Kjeldahl method (2.057, AOAC, 1980), multiplying the total nitrogen content by 6.25. Total solids were analyzed following the method M6e described by Lees (1968) and total residue analysis by APHA (1979). Approximately 20 g of sea sand (Fisher Scientific, Fair Lawn, New Jersey, USA) was weighed in stainless steel weighing dishes containing a small glass rode in each and dried at 105°C over night in a conventional oven. Dishes were cooled in a dessicator and around 5 g samples were mixed and dried at 105°C over night. Calculated the total solids from the weight gain of the dishes.

## 2.3. Results and discussion

### 2.3.1. Meat protein extraction

The mean values and Standard Error of the Mean for each of the dependent variables at high and low levels are shown in Table 2.2. Brittleness and hardness of the samples were not significantly affected by the processing variables under the conditions used in the experiment. Whiteness was significantly affected by the number of washes, concentration of salt solution and the amount of cryoprotectant. Redness was significantly affected only by the number of washes.

Because a standard procedure for chicken surimi production is not available, the conditions used in this process were adapted from that of surimi production with deboned fish. The variables taken into consideration in chicken protein extraction, i.e. number of washing cycles, concentration of NaCl solution, and mixing time, were found to have significant effects not on the texture but on the color of the chicken surimi gel. This was probably due to different protein structure associated with chicken meat. It is also possible that the gel strength of chicken surimi product is controlled by other processing variables or other factors during storage and gelling. However, since the fish surimi processing procedure was adapted, it was necessary to standardize it so that a consistent chicken surimi could be produced for the studies on texturization with rhubarb fiber.

As reviewed by Castaigne et al. (1990), denaturation of fish myofibrillar proteins during frozen storage occurs due to formation of protein aggregates, side to side from myosin monomers. Back et al. (1979) concluded that sugar and polyols stabilize proteins against heat denaturation through their effect on water structure, affecting the hydrophobic interactions that stabilize protein structure. However, at the levels used cryoprotectant did not significantly affect chicken surimi gel strength. This may be due to the fact that the range between high and low concentrations of cryoprotectant used in the experimental design was not wide enough. This variable showed an effect on the whiteness of the product. Cryoprotectant is mixed into the samples after the extraction process, during which the color pigments are removed. Therefore, the importance of this variable on the color could be ignored at this stage.

In the study carried out by Sierra et al. (1991), it was concluded that 3 washes with water:mince ratio of 4:1, concentration of salt solution of 0.2%, and mixing time of 10 min were the best conditions for a good gel strength of fish surimi.

However, in their study each of the variables was taken into consideration one at a time. Interactions of these variables which could give different results in the gel strengths were not taken into account.

In the present study, the effects of individual variables and their interactions were taken into account through the factorial experimental design. Therefore, the results obtained should be more meaningful and reliable than the one-factor-at-a-time approach used by Sierra et al. (1991).

### 2.3.2. Optimization of extraction process

Means of actual and ideal values indicated by the panel for smoothness, firmness, juiciness and the Standard Error of the Means of these values are given in Table 2.3. The evaluations given by the panel for smoothness between samples were not found to be significant ($p = 0.39$), but the firmness and juiciness were significant at p values of approximately 0.002. Overall acceptability was not found to be significant ($p = 0.34$).The ideal values for the attributes that were significantly affected (firmness and juiciness) fell towards the softer end of the scale (firmness - lower end of the scale, juiciness - higher end of the scale) as in Figure 2.4, when compared to the values given for softer sample (186) and harder sample (906). In both the attributes, the softer sample was scored closer to the 'Ideal Point' (Table 2.3).

The waste water analyses for protein content, red color and total solids are shown in Figures 2.5, 2.6, and 2.7, respectively. The results for color removal with 0.15% and 0.3% NaCl solutions through 8 washes is shown in Figure 2.6. Protein losses and the amount of total solids in washed water through 8 washes with 0.15% and 0.3% NaCl solutions are shown in Figure 2.5 and 2.7.

The washing experiment showed that protein extraction still continued after five washes, but at a greatly reduced rate. This indicated that excess washing results a low yield. However, after the fourth wash the red color pigment remained constant, irrespective of its original level in the pre-washed material and also the color in the washed water no longer changed after the fourth wash as compared to the beginning of the process.

There was a marked difference between the first and the second washes in the redness of waste water when washed with 0.3% salt solution. A gradual decrease of color was observed thereafter. There was no change in color after the 4$^{th}$ wash. In washing with 0.15% salt solution, the removal of red color in the first wash was noticeably lower compared to samples washed with 0.3% salt solution. Thereafter, the amount of color extracted by the two salt solution was not markedly different.

As shown in Figure 2.5, the protein content of waste waters at the first wash was similar for both concentrations of NaCl solutions at 0.92% and 0.82%, respectively. However, after subsequent washes, the protein loss with 0.3% NaCl solution was lower compared to that in 0.15% solution. The losses decreased gradually in both solutions, and after the 5$^{th}$ wash it was negligible. As shown in Figure 2.7, the total solid losses in 0.15% and 0.3% NaCl solutions decreased from about 3.5% in the first wash to about 1.3% level in the second wash. Thereafter, the total solid losses remained constant at about 0.5% in each wash.

## 2.4. Conclusion

Within the ranges of values of the processing variables used in this experiment, no significant effects of individual variables were detected on the texture of the gels. Therefore, it would appear that the number of washes between 3-5,

concentration of NaCl solution of 0.1-0.5%, mixing time of 5-15 min and addition of cryoprotectant mix of 9.98-12.8% do not have significant influence on the firmness of chicken surimi gel. The color of the product was affected, whiteness by the number of washes ($p=0.05$), concentration of the salt solution ($p=0.03$), and the amount of cryoprotectant ($p=0.03$). Redness was affected only by the number of washes ($p=0.02$). It could be concluded that the processing variables involved in the meat extraction process mainly affect the color but not the texture.

The results from the sensory analysis suggest that the texture of softer sample (186) was preferred by the panel when compared with the harder sample (906). The preference for softer sample was similar for all three attributes evaluated, i.e. 1) smoothness, 2) firmness and 3) juiciness. Although none of the prepared samples were ideal, the evaluation of overall acceptability indicated that the softer sample was more acceptable than the harder one. These results suggest that the consumer preferred a product which was even smoother, softer and moister than the samples presented. This leads to the conclusion that in texturization of chicken surimi with rhubarb fiber, it is necessary to adjust the processing variables in such a way as to realize a softer product.

Results presented in the figures indicate that 0.3% salt solution is a more efficient wash water than the 0.15% solution, as more pigments were extracted, and protein and total solid losses were lower than those in the 0.15% solution. The results also show that after 4 washes there was little change, either in color removal or losses of protein or total solids. However, due to the nature of the raw material (MDCM), the original color level may vary according to the batch used. Therefore, 5 washes with 0.3% salt solution should be used in subsequent protein extraction from MDCM.

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**Figure 2.1 Meat protein extraction**

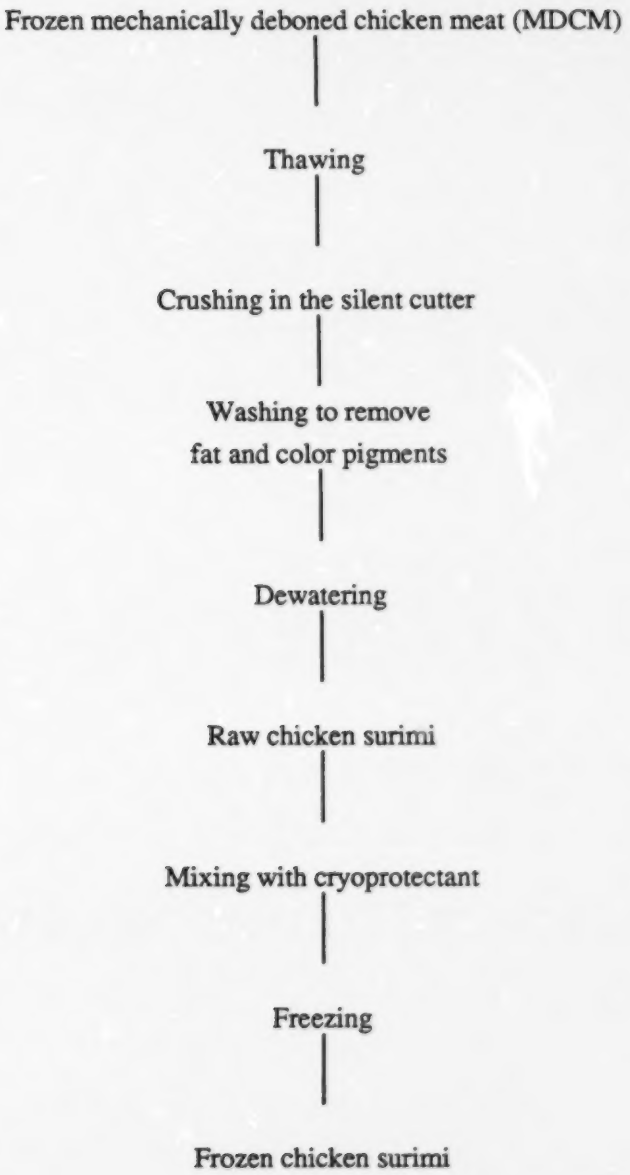
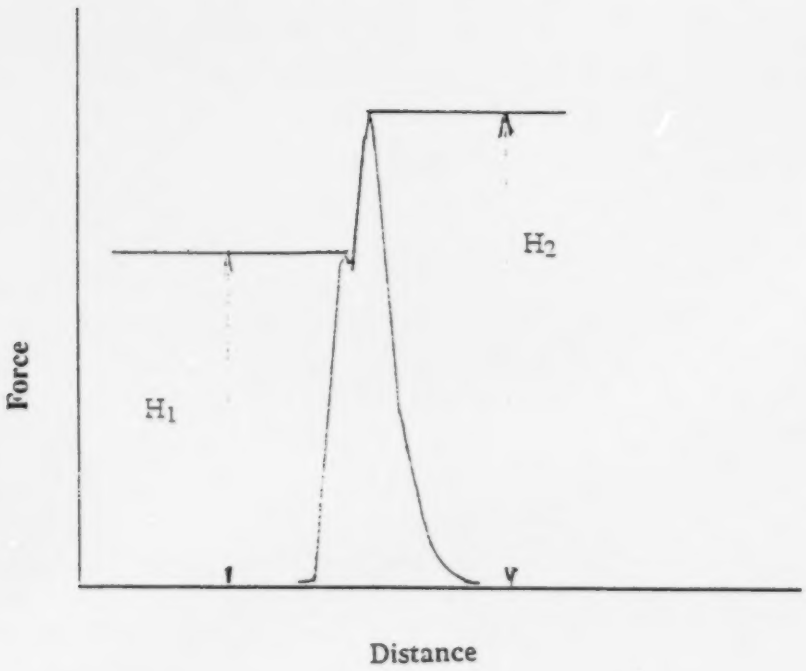


**Table 2.1 Processing variables in meat protein extraction.**

| Variable | Level | |
|---|---|---|
| | Low | High |
| Number of washes | 3 | 5 |
| Concentration of salt solution (%) | 0.1 | 0.5 |
| Mixing time (min) | 5 | 15 |
| Cryoprotectant (%) | 9.98 | 12.80 |

**Figure 2.2 Process flow chart for sample preparation**




$H_1$- Brittleness

$H_2$ - Hardness

**Figure 2.3 Typical results from texture analysis**

**Table 2.2 Mean values for texture analysis**

| Variable | Level | Brittleness (kg) | Hardness (kg) | Whiteness (L) | Redness (a) |
|---|---|---|---|---|---|
| A | 3 | 34.75 | 42.13 | 63.73 | 2.48 |
| | 5 | 33.56 | 40.75 | 65.55 | 2.30 |
| B | 0.1 % | 33.69 | 41.69 | 63.48 | 3.10 |
| | 0.5 % | 34.63 | 41.19 | 65.80 | 1.68 |
| C | 5 min | 32.06 | 39.69 | 65.13 | 2.60 |
| | 15 min | 36.25 | 42.19 | 64.15 | 2.18 |
| D | 9.98 % | 33.50 | 41.38 | 65.83 | 2.03 |
| | 12.80 % | 34.81 | 41.50 | 63.45 | 2.75 |
| SEM | | 2.44 | 1.24 | 0.42 | 0.23 |

A - Number of washing cycles

B - Concentration of salt solution

C - Mixing time

D - Amount of cryoprotectant

SEM - Standard error of the mean

**Table 2.3 Mean values for sensory attributes**

| Sensory attribute | Ideal | Sample | | SEM | Pr. |
|---|---|---|---|---|---|
| | | harder (906) | softer(186) | | |
| Smoothness | 5.67 | 4.79 | 5.42 | 0.46 | 0.3942 |
| Firmness | 4.57 | 6.50 | 5.47 | 0.35 | 0.0022 |
| Juiciness | 5.20 | 3.23 | 4.41 | 0.36 | 0.0018 |
| Overall acceptability | | 4.61 | 5.29 | 0.49 | 0.3431 |

SEM- Standard error of the mean

Pr.- Probability

**Figure 2.4 Sensory profile used in texture optimization**

**Questionnaire**

Name: ..... Date: .....

Evaluate the texture of given samples, and the ideal for each of the attribute by a non oral method, by following the described procedure. **Indicate the rating** by marking vertical lines on the scale labeled with the code number. Also mark the **'Ideal Point'** with an **"I"**

**Smoothness:** Cut samples with a knife and study the smoothness of the cut surface.

|_____|
Too rough Extremely smooth

**Firmness:** Feel samples with fingers.

|_____|
Too soft Too hard

**Moistness:** Break and press samples with fingers.

|_____|
Too dry Too moist

**Overall acceptability:** The overall rating for samples by evaluating above attributes.

|_____|
Extremely unacceptable Extremely acceptable

Comments:.....

.....

.....

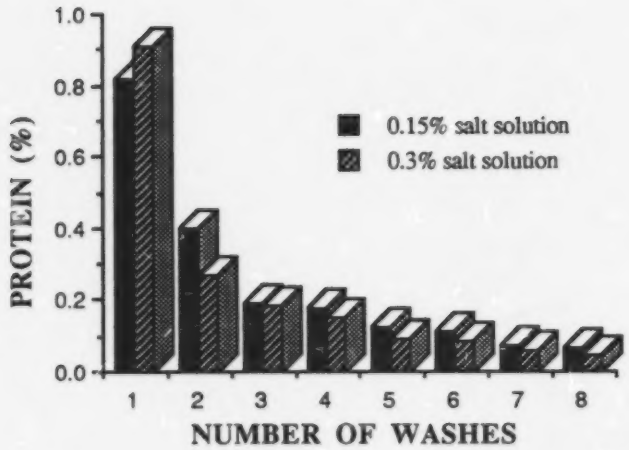



**Figure 2.5 Protein in waste water**

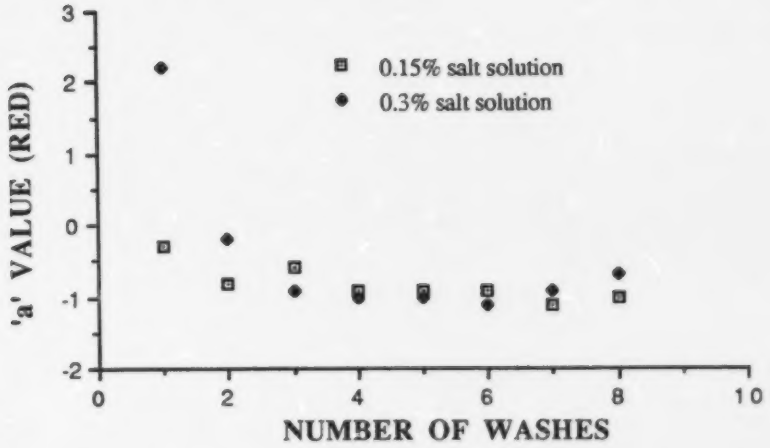



**Figure 2.6 Color in waste water**

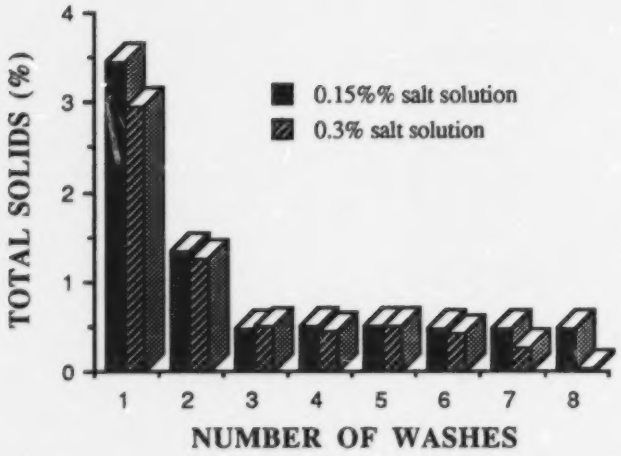



**Figure 2.7 Total solids in waste waster**

# Chapter 3. Effect of Rhubarb Fiber on Chicken Surimi Gel

## 3.1. Introduction

Preparation of 'kamaboko' (surimi based products) may be considered as a process that links molecular denaturation and association events with textural and water holding properties of raw or frozen surimi. Thermally induced unfolding and aggregation of muscle proteins forms a matrix that provides processed meats with their texture (Foegeding, 1988). The general process of rigidity development in myosin gelation begins at 40-50°C, increases to 60°C, then remains relatively constant from 60-70°C (Ishioroshi et al., 1979; 1981, Wicker et al., 1986; Yasui et al., 1979).

In a study conducted to determine the effects of the internal temperature attained during the cooking of chicken meat loaves on salt-soluble protein extractability, loaf binding strength and cooking loss, Acton (1972) reported that instantaneous heating of thin tissue slices of poultry meat significantly decreased sarcoplasmic and myofibrillar protein extractability. He also reported that at 43°C, there was a 25.9% decrease in extractability and at 55°C, a reduction of 60.9% of salt soluble proteins.

The transitions within the 40-70°C range depend on the source of myosin, i.e. chicken, fish or rabbit (Foegeding, 1988). The major increase in rigidity development begins in the range of 50-58°C in the more complex systems of comminuted pork, beef and turkey, myofibrils, surimi and frankfurters (Foegeding and Ramsey, 1987; Patana-Anake and Foegeding, 1985; Samejima et al., 1985).

Surimi gels which were cooked by heating at 40°C for 1h, then at 90°C for 15 min, have greater failure shear stress and shear strain values than those cooked at 90°C for 15 min (Montejano et al., 1984). This is generally referred to as the

"setting phenomenon" and is a direct example of how processors can use heat to direct protein-protein interactions to produce variations in texture (Foegeding, 1988).

In this research, chicken protein extracts were subjected to temperature-time treatments to form 'chicken surimi gel' in order to obtain an optimum temperature-time combination to be used in the subsequent texturization process. The meat gel has to be firm so that it gives strength to the end product. The product also has to be soft and moist to satisfy consumer preference. While taking these factors into consideration, the effect of amount of rhubarb fiber added to the chicken surimi on its texture was investigated.

## 3.2. Materials and methods

At this stage of experimentation rhubarb fiber was taken into consideration as a variable in the process. This variable was included in the experimental designs. Gelling was experimented using two experimental designs. After analyzing the results from the first (fractional factorial $2^{4-1}$) the second experiment was designed (factorial $2^3$). The $2^{4-1}$ fractional factorials and $2^3$ factorials were analyzed using analyses of variance. Sources of identified variations were computed using Statistical Analysis System (1985). Standard errors of the mean of each product attribute were computed using the pooled estimate of error variation.

### 3.2.1. Rhubarb fiber extraction

Rhubarb stalks were supplied by domestic rhubarb growers in Edmonton, Alberta. Long fiber extracted from either fresh or frozen rhubarb stalks and stored under frozen condition (at -30°C) was used as the fiber source at the initial stage of the study. The cell cementing materials and juice were removed by blanching, pressing and combing the stalks. The remaining long fiber was washed in cold water and stored at -30°C until used.

Fiber was extracted from both pink (McDonald) and green (German wine) rhubarb varieties following the procedure suggested by Ooraikul and Liang (1990) as shown in the process flow chart (Figure 3.1). In the fiber extraction, frozen rhubarb stalks were thawed at room temperature (~ 15°C) for 2 hr, and then steamed in a blancher for 1 min 20 sec at 100°C. A thin layer of stalk skins was removed, and the stalks were beaten with a rubber mallet to break up cementing material between fiber strands.

Furthermore, the material was blanched to soften the tissue sufficiently, but not excessively, to weaken the fibers, so that fiber strands may be separated cleanly from one another while retaining appropriate amount of pectin "coating" for desirable chewing characteristics. Frozen stalks were softened by freezing and thawing (frozen stalks left at room temperature- ~15°C, for ~2 h) normally required less time for blanching. Combing was done by pinning one end of the pressed stalk down and gently combing it with a hair comb through the other end, then reversing it, starting with a coarse comb and followed by finer ones. This removed much of the pectic substances binding the fibers as well as short broken fibers.

### 3.2.2. Mixing rhubarb fiber with meat extracts

A uniform distribution of fiber in meat batter was difficult to obtain when the fiber was mixed directly into the extracted meat protein. The length of the fiber was also a contributing problem. Therefore, the long fiber was cut into approximately 5 cm pieces. To obtain a more uniform mixture both MDCM extract and cut fiber were first made into thick suspensions with water and then mixed in a Hobart mixer. The mixed suspension was strained through a plastic strainer and further dewatered in a basket centrifuge which was lined with a coarse filter paper.

Rhubarb fiber contained about 90% moisture and extracted protein about 72%. After mixing and dewatering the moisture of the final product was about 79%.

### 3.2.3. Gelling experiments

Gelling was carried out in two sets of experiments. Products from the first set of gelling experiments were analyzed for texture prior to proceeding with the second set of experiments. Processing variables included in the first experiment were: 1) chopping time with dry salt (NaCl), 2) gelling temperature, 3) gelling time and 4) amount of rhubarb fiber added as a percentage of the weight of extracted chicken protein sample. The amount of cryoprotectant has been reported as a significant variable in fish surimi production (Lee, 1986; Ablett et al., 1991; Sierra et al., 1991), but did not show significant effect on the texture of chicken surimi (Chapter 2). Therefore, the amount of cryoprotectant was maintained at a constant level of 11.4% in the gelling experiments. The standardized procedure for chicken protein extraction was used for sample preparation in gelling experiments. The process therefore consisted of 5 washing cycles with 0.3% salt solution each with 7 min mixing time, and 11.4% cryoprotectant by weight of extracted meat protein added.

## 3.3. Experimental procedure

Preliminary gelling trials were carried out by heating the chicken surimi samples in incubators at 37° and 60°C for different lengths of time. Texture of the samples was measured with ASTM penetrometer at 15 min intervals to study the changing pattern of the gel strength. Optimum gelling temperature and gelling time were decided based on the results of texture measurements. The low and high levels for chopping time were chosen to minimize possible heat generated that may occur during the process, which may have an adverse effect on the protein composition of

meat. Process flow chart for preparation of samples for gelling experiments is shown in Figure 3.2.

### 3.3.1. Gelling experiment #1

A half-fractional factorial experimental design ($2^{4-1}$) was used in this study (Box, Hunter and Hunter, 1978). Each experimental unit was coded with a three digit random number. The low and high levels of processing variables included in the design are shown in Table 3.1.

Rhubarb fiber was incorporated as weight percentage of extracted meat protein and cryoprotectant was then mixed in as weight percentage (11.4%) of combined weights of meat protein and fiber. Samples were vacuum packaged in polyethylene bags using Multivac (Type AG-500, Made in W. Germany) and stored at -30°C over night. Thawing was carried out for three h at room temperature within the vacuum packaged bag. Dry salt (NaCl, Sifto) was mixed at 3% of the total weight of the sample in a household type food processor. The mixtures were filled into 10 mL disposable beakers and sealed with parafilm to prevent case hardening on the surface. Gelling process was carried out at designated temperatures, for the periods of time as indicated in the experimental design. Two still-air incubators were used in the gelling process in which the temperature was set at 30° and 50°C.

### 3.3.2. Gelling Experiment #2

In the next gelling experiment (Gelling Experiment #2) both gelling temperature and gelling time were applied at increased levels. Although the presence of fiber had not shown a significant effect on texture, it was also included as a variable in the design to study its effect under new temperature - time combinations. Chopping time was maintained at a constant level (3 min). Processing variables and their levels included in gelling experiment #2 are shown in Table 3.2.

A full factorial experimental design ($2^3$) was used in this experiment (Box, Hunter and Hunter, 1978). Each of the eight experimental units was coded with a random number and the order of experimental units were randomized. Products of the experiments (chicken protein gels) were analyzed using the same procedure as in gelling experiment #1.

### 3.3.3. Analysis of the product

Samples were removed from the incubators and allowed to reach room temperature. Meat gels were released from the disposable beakers, which were used to hold the samples during the gelling process, and tested for texture using ASTM grease penetrometer ('Precision' penetrometer, Precision Scientific Co., Chicago, USA). The cone shape probe (45g size) was used in analyzing the texture. Depth of penetration during the first 5 seconds, from the time of releasing the shaft with the probe was measured. The depth of penetration may be taken as a rough indication of hardness.

## 3.4. Results and discussion

The mean texture values and Standard Error of the Mean obtained from results of the gelling experiment #1 are shown in Table 3.3. The mean values were calculated from the values for the depth of penetration through the sample. Gel strengths of the samples from eight experimental runs were grouped into low levels and high levels of each of the processing variable. The mean values shown are calculated from four replicates grouped under high and low levels of each variable measured in the texture analyzing instrument.

At the levels included in the first gelling experiment it was observed that the effect of chopping time ($p=0.91$) and fiber content ($p=0.88$) on the texture of

the product were not significant. Gelling temperature and gelling time had effects on texture of the product at levels of p=0.07. Chopping time may have an effect on the texture if the difference between the high and low levels was larger. However, due to the small size of the sample processed (approx. 1kg), it was not practical to use the silent cutter at this stage and, instead, a small household food processor (Food Processor, Cuisinart DLC-8PLUS) was used. In the small food processor, a chopping time longer than 180 seconds generated excessive amount of heat which would denature and, hence, prematurely gel the protein. In a large batch when the silent cutter is used the effect of chopping time may, therefore, be different from that in the household processor. Nevertheless, under the conditions used in this experiment the chopping time did not significantly affect the texture of the product. The gelling time between 30 min and 6 h and gelling temperature between 37 and 50°C showed similar effect on the texture. Rhubarb fiber between 5 and 10% did not show significant effect on the texture.

According to the primary objective of the research project, rhubarb fiber was the most important variable in the process. However, at the concentrations between 5 and 10% fiber showed no significant effect on the product texture. Therefore, to study the effect of fiber on the texture its concentration in the mixture had to be raised. Also, the levels of other variables in the experiments were adjusted so that their effects would not overshadow that of fiber. Therefore, chopping time which was not a significant factor was fixed at 180 s. Since the addition of fiber had been observed to soften the product texture, and since lower gelling time and temperature also produced softer product the ranges of gelling time and temperature were, therefore, raised so as to allow the softening effect of fiber show more clearly.

Based on the results from gelling experiment #1, modifications to the range of the values of the processing variables were made in gelling experiment #2. In

experiment #2, gelling temperatures were changed to 43 and 55°C, and gelling times to 2 and 6 h at low and high levels. The difference between the levels of fiber was increased by raising the higher level to 15%. These changes were aimed at generating a chicken surimi gel which would show some significant effect of rhubarb fiber on its texture.

Results from gelling experiment #2 are shown in Table 3.4. Gelling temperature was not found to be significant at the range used in the experiment. Gelling time showed a significant effect at $p \leq 0.05$ and fiber level showed an effect at $p \leq 0.08$.

## 3.5. Conclusion

Under the conditions used in Gelling experiment #2 1) gelling time (between 2 and 6 h) and 2) amount of fiber incorporated (between 5 and 15%), showed modest effects on the texture of the product. Gelling temperature was not found to be significant within the range used (43-55°C). The second gelling experiment showed that the effect of rhubarb fiber on chicken surimi produced under the proposed method could be observed when the amount of fiber added was at least 15% of the total weight of the surimi and gelled within the temperature range of 43 to 55°C and time of 2 to 6 h.

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**Figure 3.1 Process flow chart for rhubarb fiber extraction**

**Figure 3.2 Process flow chart for chicken surimi gelling**

**Table 3.1 Levels of processing variables in Gelling Experiment #1**

| Variables | Level | |
|---|---|---|
| | Low | High |
| Chopping time (sec) | 30 | 180 |
| Fiber content (%) | 5 | 10 |
| Gelling temperature (°C) | 37 | 50 |
| Gelling time (min) | 30 | 360 |

**Table 3.2 Levels of processing variables in Gelling Experiment #2**

| Variable | Level | |
|---|---|---|
| | Low | High |
| Fiber level (%) | 5 | 15 |
| Gelling temperature (°C) | 43 | 55 |
| Gelling time (h) | 2 | 6 |

**Table 3.3 Mean values for texture analysis - Gelling Experiment #1**

| Variable | Level | | Depth (mm) | Pr. |
|---|---|---|---|---|
| Chopping time (sec) | Low | 30 | 21.33 | 0.91 |
| | High | 180 | 21.65 | |
| Fiber content (%) | Low | 5 | 21.25 | 0.88 |
| | High | 10 | 21.73 | |
| Gelling Temperature (°C) | Low | 37 | 25.38 | 0.07 |
| | High | 50 | 17.60 | |
| Gelling Time (min) | Low | 30 | 25.45 | 0.07 |
| | High | 360 | 17.53 | |
| SEM | | | 1.59 | |

SEM- Standard Error of the Mean

Pr.- Probability

**Table 3.4 Mean Values for texture analysis - Gelling Experiment #2**

| Variables | Level | | Depth (mm) | Pr. |
|---|---|---|---|---|
| Fiber level (%) | Low | 5 | 15.36 | 0.08 |
| | High | 15 | 20.33 | |
| Gelling temperature (°C) | Low | 43 | 18.99 | 0.30 |
| | High | 55 | 16.57 | |
| Gelling time (h) | Low | 2 | 20.75 | 0.04 |
| | High | 6 | 14.80 | |
| SEM | | | 1.44 | |

SEM- Standard Error of the Mean

Pr.- Probability

# Chapter 4. Sensory Evaluation of Fibre-incorporated Chicken Surimi

## 4.1. Introduction

Texturization of surimi-type products with long vegetable fiber is a new development in food processing never previously reported. There is no texture standard that these products could be compared to. Therefore, it was necessary to obtain guidance to desired texture for these types of products through the use of a sensory panel to evaluate samples of widely differing textural characteristics and express their preference.

Texturization of surimi products to gain a better gel strength has been studied in a number of research projects (Enriquez et al., 1990; Hujita and Makinodan 1990; Chung and Lee, 1990; Rodger, 1992). Hujita and Makinodan (1990), in an attempt to improve the texture of fish protein gel by incorporation of edible mushroom, Judas' ear (*Auricularia auriculajudae* (Fr.) Quel) to the gel, found that the texture of the gel was degraded by hydrolysis of myofibrils by the mushroom proteinase. Enriquez et al. (1990) reported improved gel strength as a result of adding ingredients such as egg white, starch, chemical seasoning, vegetable oil, etc. The meat-like texture was obtained only by mechanical means after improving the gel strength with the aid of other ingredients (Lee, 1986; Enriquez et al., 1990; Rodger, 1992).

In an attempt to increase the utilization of surimi, Carpenter et al., (1990) investigated the effect of surimi protein on pasta products. He reported a decrease in firmness of cooked pasta with an addition of 10%, 20% and 30% surimi. Information on ultra-structures of the pasta-surimi mix corroborated these finding.

Sensory evaluations have been commonly used in studying the gel strength of products (Hultin et al. 1984). However, little attention has been paid to the

consumer preference of the texture of a surimi based product. Texture modification using vegetable fiber also is a new area of research for these types of products. In this project, texture modification of chicken surimi using rhubarb fiber was studied using a sensory panel as a tool to obtain a desired texture. A Safeway chicken nugget (product of Lilydale Poultry Processing Company, Edmonton, AB) was used as a reference substance.

As observed earlier, fiber at a minimum concentration of 15% had a modest effect ($p=0.08$) on the texture of the product within the ranges of gelling time and temperature used in Gelling experiment #2. To be able to observe a more significant effect of fiber it was decided to increase the amount of fiber to 20% of the weight of extracted meat samples and to compare the results with samples which had no fiber. Preference of the level of texture and acceptability according to sensory evaluation of the product when fiber is incorporated were also studied. Texturization experiments were carried out using the chicken surimi prepared with the standardized procedures obtained from the previous experiments (meat extraction and gelling experiments). Products were analyzed through a series of sensory evaluations. The conditions followed in sample preparation for sensory evaluations are shown in Table 4.1. Instrumental values were obtained parallel to subjective results. The objective of this operation was to compare and to justify the order of softness of the products reported by the sensory panel.

## 4.2. Sensory Evaluation

Under the processing conditions used in Gelling Experiment #2, fiber had shown a modest effect ($p=0.08$) on the texture of the product. However, sensory attitude towards the texture modified with the aid of rhubarb fiber still needed to be determined. It was important to identify the direction and quantitative values for texture improvement for further studies. In the area of sensory evaluation, panelists

were used as the 'analytical tool' with an ability to discriminate the texture of the products. Chicken nuggets purchased from Safeway, Edmonton, Alberta (Lilydale product) were used as standard samples in the evaluations. These chicken nuggets were used to provide the sensory panel with an idea of the texture of a similar poultry product already available in the market.

Sensory profiles were designed and a rating/scoring test (N.Z.I.F.S.T., 1986) was utilized in this evaluation. This profile consists of a line of specific length with anchor points at each end and usually, but not necessarily, with a third anchor point in the middle. One word or expression is placed at each anchor point. The assessor places a vertical mark across the line at the point which best reflects the magnitude of the particular attribute. This method produces an interval scale in which there are an infinite number of different positions on the line. In the present study, to avoid the variations that could occur due to different lengths used, the ratings were converted into a fixed length taking the minimum and maximum as 0 and 9.

The procedures to be used in analyzing the product for each of the attributes were explained in the questionnaires. Texture measurements were also obtained using a penetrometer constructed locally, which was equipped with a vertical shaft operated by a speed reducer motor connected to an adapter (Model SL14, Minarik Electric Company, Lose Angeles) which controlled the direction of the shaft movement. The response was recorded on a strip chart.

### 4.2.1. Preparation of Samples

Samples were prepared on the day prior to the sensory evaluation. The flow chart for sample preparation is shown in the Figure 4.1. Ten mL disposable beakers were used in the gelling process to obtain standard size samples for sensory

evaluation. Deep frying was carried out to ensure the wholesomeness of the product tasted by the sensory panel.

For one session of sensory evaluation four batches of chicken surimi were processed following the procedure shown in the Figure 2.2. Two batches of extracted meat protein were mixed with rhubarb fiber prior to mixing with cryoprotectant. Samples were stored at -30°C and then thawed at 4°C overnight and at room temperature for 3 h. Dry salt (Sifto) was mixed in the silent cutter with each batch at 3% of the total weight of the extracted chicken protein. Extracted chicken protein samples were loaded into the silent cutter and while the cutter was operating, dry salt was sprinkled through the opening of the mixing bowl. Mixing was stopped after 5 min and the mixture was scrapped together with a plastic spatula and allowed to mix for a further 5 min. Samples were vacuum packaged in vapor impermeable cellophane bags. Size of the samples presented to the panel was standardized by using 10 mL disposable beakers for sample preparation. Disposable beakers were filled with the meat batter and covered with parafilm to prevent surface hardening. Heat treatment was carried out at 55°C for 5 h in an incubator. After removing from the incubator, samples were allowed to cool to room temperature. Meat gels were released from the disposable beakers, wrapped with bread crumbs and stored in the cooler (at 4°C) for 7 to 8 h. Coating with bread crumbs was necessary to make the products similar to the standard sample, chicken nuggets, used in the sensory evaluation. Coated chicken surimi gel samples were deep fried in Crisco vegetable oil 30 min prior to the evaluation in order to ensure the safety of the product for oral testing.

### 4.2.2. Sensory Evaluation Procedure

Two sessions of sensory evaluations were held. In the first session 16 panelists were available for the evaluation. In the second session 14 panelists

participated. In each session two sets of evaluations were carried out, first in the morning from 10.30 till 11.30, second in the afternoon from 2.30 till 3.30. In both the morning and afternoon testing, evaluations were carried out in three repetitions. Repetitions were done with the same batch of processed samples but coded with different random numbers.

The panel was presented with a plate of coded samples of chicken surimi gels, with and without fiber, and commercial product together with the questionnaire. Second and third plates were presented in similar manner, but with different code numbers. A score sheet was also provided. Panelists were asked to evaluate the texture of samples given to them.

#### 4.2.3. Testing Environment

All the sensory evaluations were carried out in the sensory panel room in the Food Science Department, University of Alberta. Preparation of samples was carried out in the kitchen adjacent to the sensory panel room. The sensory panel room accommodates ten panelists at a time and is equipped with individual booths for each panelist. Evaluation was made under lighted environment.

#### 4.2.4. Setting up the Questionnaire

Although the sensory panel used was not trained for this particular product evaluation, every panelist was thoroughly acquainted with sensory evaluation of different types of food products. Therefore, a broader spectrum of product attributes which included three attributes namely, springiness, hardness and chewiness, were included in the first session to identify the attributes that could be analyzed by this panel. This initial study was also aimed at drawing the panelists' attention to attributes that could give an idea about the mouthfeel of the product. In the questionnaire given in the first session, it was also requested to mark the 'Ideal

Points' (Beausire and Earle, 1986) for each of the attributes. This 'Ideal Point' was to be decided by each of the panel members according to her/his own perception. The panelists were advised to study the product and to indicate the direction that each of the attributes could be improved by marking their 'Ideal Points' on the scale. The attributes to be analyzed in the first session were: springiness, firmness and chewiness. Springiness was to be evaluated by pressing the samples with two fingers, firmness was to be evaluated by cutting with a knife and the chewiness was to be evaluated by chewing in the mouth. In one session there were six evaluations - three repeats in the morning and three in the afternoon - and the panelists were allowed to have a floating 'Ideal Point' (mark the ideal according to the individual perception). The sensory profile used in this session of evaluation is shown in Figure 4.2. In the second session springiness was removed from the sensory profile as it was observed that either the treatments were not significant on this attribute or the significance was not detectable due to hard coating on the sample. Analysis of the score sheets in the second session was carried out following the same procedure as for the first session. The questionnaire used in session two is shown in Figure 4.3.

#### 4.2.5. Experimental Design

Input information for analysis in the first session were blocks - 2 (morning and afternoon), treatments - 4 (three treatments and ideal values), analyzed by 16 panelists, in three repeats in each of the blocks. This corresponds to 96 values. The mean values were then calculated from the 96 observations. In the second session two of the panelists were dropped from the analysis due to incomplete scoring sheets. Input information for the second session were blocks - 2, treatments -3, analyzed by 12 panelists in three repeats. The mean values obtained were from 72 observations for each of the treatments.

## 4.3. Analysis of score sheets

Scoring lines were measured from the left ends to the marked vertical lines for the coded samples for three treatments, 1) with fiber, 2) with no fiber, 3) commercial product and 4) the ideal levels indicated by the panelists. Results, measured to the nearest whole number on the 9-division scoring line, were reported for springiness, hardness and chewiness. The results were categorized into two blocks: morning and afternoon. Each of the blocks was grouped into three repeats (three repeated evaluations in one block).

Data were analyzed by analysis of variance using General Linear Models (GLM) procedure (Statistical Analysis System, Inc. 1985. SAS User's Guide: Statistics. Version 5. SAS Institute, Inc. Cary, NC.). Questionnaires were analyzed for scores of three treatments, 1) fiber incorporated samples, 2) samples with no fiber and 3) commercial product. 'Ideal Points' indicated by the panelists for each attribute also were analyzed in the first session of evaluation and the mean values of these were used as fixed 'Ideal Points' in on the scoring lines in the second session.

## 4.4. Objective analysis

Parallel to sensory evaluation, texture of the products was also measured using the texture meter. Equal size samples were taken from the three products, 1) with fiber, 2) without fiber, and 3) commercial product. The texture meter was calibrated with an 800 g weight to give a full width of a 0-100 scale on strip chart, where 0 represented zero force and 100 represented 800 g force. A probe with 3 mm diameter was used for penetration. Samples were placed on a plastic ring which was centered on the strain gauge of the texture meter. Strip chart speed was adjusted at low setting on the control panel. Speed of the penetration of the probe was set at the lower level. Each sample was measured in duplicate.

**4.5. Results and discussion**

Mean values were calculated for each of the attributes evaluated under three treatments. The means of the ideal value recommended were also calculated for each of the attributes. The mean values and standard error of the mean are presented in Table 4.2. Springiness did not show a significant effect in sensory analysis. Hardness and chewiness both had significant effects at p=0.03 levels. Mean scores were compared with the mean ideal values. The deviation of the scores from the ideal values in the first session are shown in Table 4.3.

In the analysis of the sensory scoring sheets in the first session, it was observed that springiness was not identified by the panelists as being related to the texture of the product. Therefore, evaluation for this attribute was removed from the sensory profile for the second session. The attributes included in the sensory profile in this session were hardness and chewiness.

Assuming the 'Ideal Points' were obtained from a consumer panel, the mean ideal value for sensory attributes was calculated. During the second session, the 'Ideal Points' were fixed at the mean value. In the sensory profile presented for the second session, the 'Ideal Points' were marked on the scale for all the attributes.

In sensory evaluation, chewiness was considered as the critical parameter to be analyzed. As shown in the Table 4.3 the mean value of scores obtained for this parameter for samples with fiber is very similar to that of the ideal value suggested by the panel. As the results obtained were from a subjective test method (sensory evaluation) this level of similarity could be inferred as no different from the ideal value. Although hardness deviated from the ideal value, the product with fiber had the least deviation when compared with the product with no fiber (Table 4.3). Therefore, it appeared that the hardness of the product with fiber, which

was found to be softer than the product with no fiber according to both subjective and objective analysis, was preferred by the panel.

The mean values of hardness and chewiness and the standard error of the means for the second session of sensory evaluation are shown in the Table 4.4. Hardness showed a significant effect at p=0.01 and chewiness showed a modest effect of p=0.06. Both sensory evaluation sessions had the same trend of preference among products with fiber, products with no fiber and the commercial product. Deviations from the ideal value in the second session are shown in Table 4.5. The higher level of deviation of scores in the second session may have resulted from the differences in the raw material between batches. The mean ideal values for the second session were fixed at the levels indicated in the first session of the analysis.

The pattern of the curves obtained on a strip chart in analyzing the texture of products with fiber, without fiber and the commercial product is shown in Figure 4.4. On a scale of 0 - 100, samples with fiber showed the lowest hardness, followed by the commercial product and the samples with no fiber having the highest hardness. The commercial product clearly indicated two peaks where the first peak represented the hardness when cut through the coating and the second peak when cut through the sample. Samples with no fiber showed a hardness which was almost twice that of samples with fiber. Where hardness is concerned, both sensory evaluation and instrumental analysis gave the same trend, i.e. that products with no fiber > commercial product > products with fiber.

## 4.6. Conclusions

Given the results obtained from the sensory evaluation, texture modification of chicken surimi using rhubarb fiber appears to be accepted by the sensory panel. The products whose texture was modified with rhubarb fiber was

preferred by the panel. Objective analysis supported the sensory results to the effect that the texture of the fiber-incorporated samples was softer than that of the products with no fiber in them. This softness appears to correlate with the improved chewiness of the product.

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**Table 4.1 Experimental conditions in sample preparation for sensory evaluation**

| Parameter | Level |
|---|---|
| Number of washing cycles | 5 |
| Concentration of salt solution (%) | 0.3 |
| Mixing time with salt solution (min) | 7 |
| Amount of cryoprotectant (%) | 11.4 |
| Chopping time with dry salt (min) | 5 + 5 |
| Gelling time (h) | 6 |
| Gelling temperature (°C) | 55 |

**Figure 4.1 Sample preparation procedure for sensory evaluation**

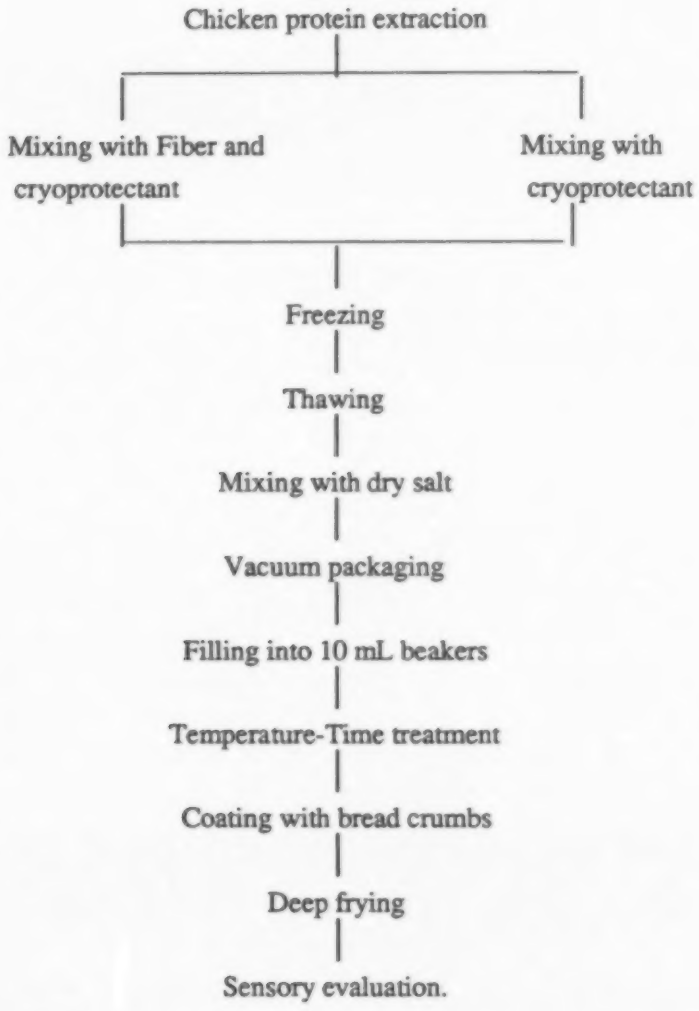


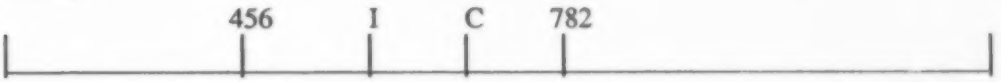
**Figure 4.2 Sensory profile used in the first session**

**Questionnaire**

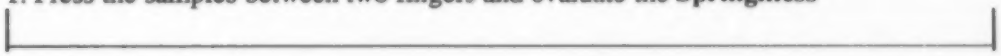
Evaluate the given coded samples of preformed chicken products for the texture and compare with the given commercial product. Mark the level of each of the attribute with a vertical line on the horizontal scale and label with the code numbers for the samples and commercial product with **'C'**. Also mark the 'Ideal Point' and label with **'I'**.

****Make sure that each of the horizontal line is marked with four vertical lines labeled each either with C, I or sample codes.**

**example:**

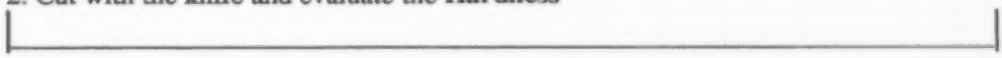



1. Press the samples between two fingers and evaluate the **Springiness**



very hard — too soft

2. Cut with the knife and evaluate the **Hardness**



too tough — too soft

3. Chew for the mouthfeel (**chewiness**)

very hard — very soft

Name:

Date:

**Figure 4.3 Sensory profile used in the second session**

**Questionnaire**

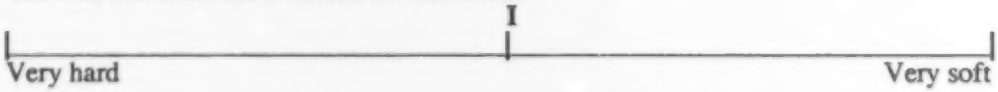
The ideal levels for hardness and chewiness are marked on the scales, and labeled with an **'I'**.

Evaluate the given coded samples and the commercial product with reference to the 'Ideal Points and indicate the level of hardness and chewiness with a vertical line for each of the samples on the horizontal scale.

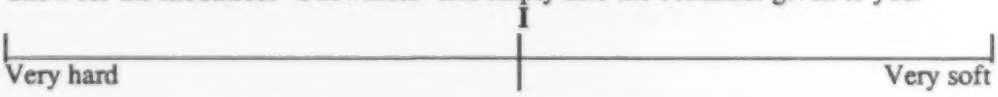
****Make sure that each of the horizontal line is marked with three vertical lines labeled each either with 'C' or sample code.**

Sample codes: **389 741**
Commercial product: **C**

Cut with the knife and evaluate the **Hardness.**




Chew for the mouthfeel -**Chewiness-** and empty into the container given to you.




**Name :** ..... **Date:** .....

**Table 4.2 Results from the sensory evaluation - session one**

| Treatment | Springiness | Hardness | Mouthfeel (Chewiness) |
|---|---|---|---|
| Ideal | 4.28 | 4.37 | 4.53 |
| With Fiber | 4.15 | 3.93 | 4.45 |
| With no Fiber | 3.67 | 3.41 | 3.62 |
| Commercial product | 4.37 | 4.81 | 5.24 |
| SEM* | 0.22 - 0.24 | 0.16 - 0.17 | 0.18 - 0.19 |

* - unequal number of observations, smallest and largest SEM are given.

**Table 4.3 Deviation from the ideal value in sensory evaluation - session one**

| Treatment | Springiness | Hardness | Mouthfeel (Chewiness) |
|---|---|---|---|
| With fiber | -0.13 | -0.44 | -0.08 |
| With no Fiber | -0.61 | -0.96 | -0.92 |
| Commercial Product | +0.09 | +0.88 | +0.71 |

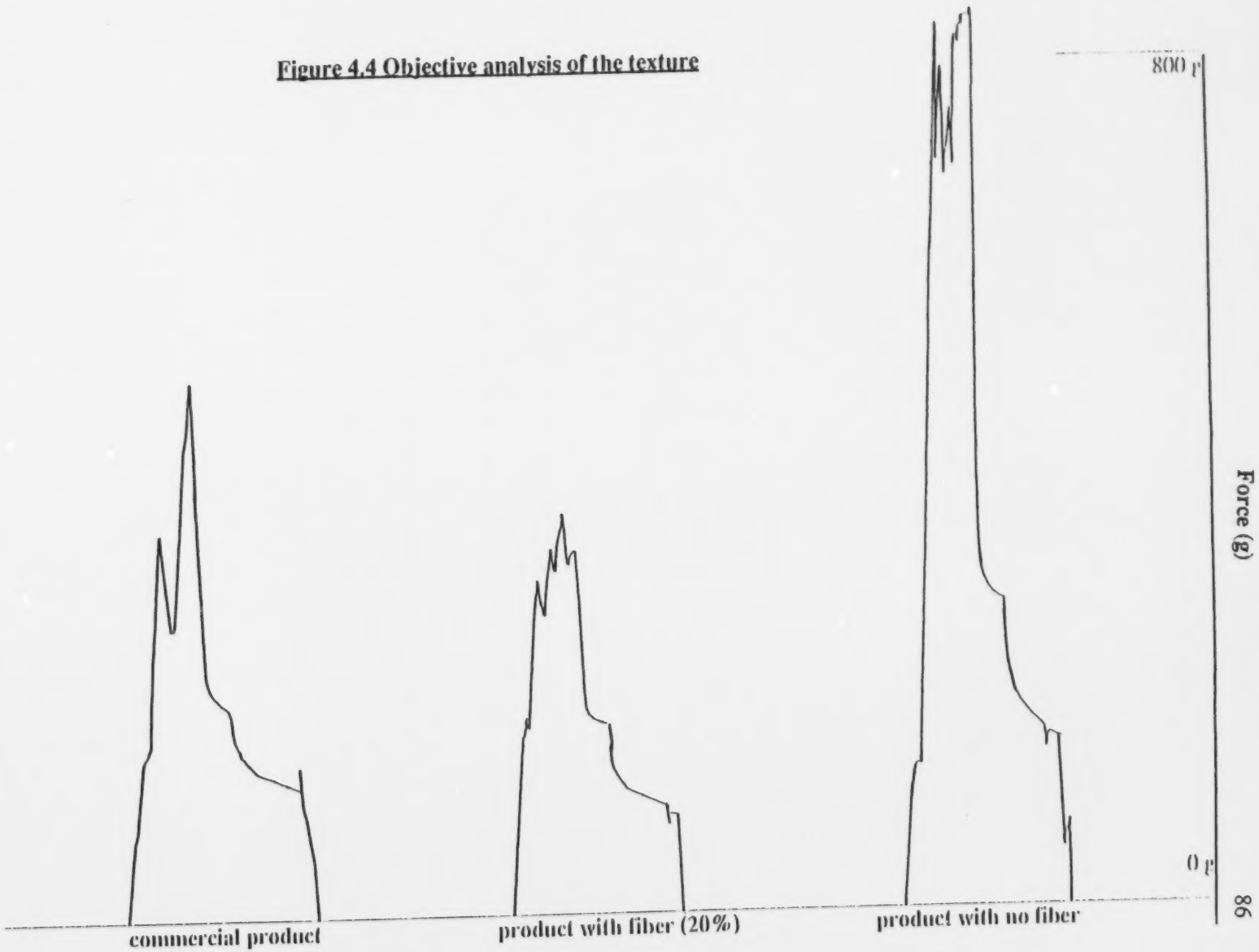
**Table 4.4 Results from the sensory evaluation - session two**

| Treatments | Hardness | Mouthfeel (Chewiness) |
|---|---|---|
| With Fiber | 3.63 | 4.02 |
| With no Fiber | 3.11 | 3.40 |
| Commercial Product | 3.33 | 3.71 |
| SEM | 0.028 | 0.079 |

**Table 4.5 Deviation from the ideal value in sensory evaluation - session two**

| Treatment | Hardness | Mouthfeel (Chewiness) |
|---|---|---|
| With Fiber | -0.7422 | -0.5108 |
| With no fiber | -1.2570 | -1.1352 |
| Commercial Product | +1.0368 | +0.8232 |

**Figure 4.4 Objective analysis of the texture**



# Chapter 5. Chemical and Physical Properties of the Product

## 5.1. Introduction

The growing demand for surimi-based shellfish analog products has resulted in continued efforts to improve the textural quality of these products, e.g., by addition of gel-forming ingredients such as starch and protein. Salt soluble protein is the most important component in surimi owing to its functional properties such as water-binding and gel-forming ability. These properties contribute to the textural acceptability and the physical stability of formulated protein gel-based products. Understanding of the functional behavior of added ingredients in formulated food systems such as surimi-based products is important for the optimization of product quality.

Chung and Lee (1990) investigated the relationships between physicochemical properties of nonfish protein and textural properties of the nonfish protein-incorporated surimi gel. In his study, the microstructure as affected by adding nonfish proteins (soy protein isolate, milk protein isolate, egg white, and wheat gluten at 2, 4 or 6%) and 6% crystalline sorbitol was investigated. The objective was to obtain information to understand what caused differences among nonfish proteins in their effects on gel-forming and textural properties of surimi. He concluded that nonfish proteins not only reduced texture hardening during frozen storage, but also modified texture during cooking as they underwent thermal gelation specific to each protein used.

As reported by Barbut and Gordon (1991), the functionality of proteins is directly dependent on their conformation within a given environment. Even small changes in environmental conditions can lead to large differences in protein conformation. He also reported that several protein-protein and protein-lipid

interactions occur prior to cooking, due to combination of intrinsic and environmental factors which affect the microstructure and texture of the cooked product.

Turning to the present research, the effect of rhubarb fiber on the texture of the end product was studied in the preceding experiments. Although the experiments showed that fiber contributed to the improved chewiness of the product, the optimum in amounts of added fiber that could result in an acceptable product was not yet studied. Different fiber levels from 5% to 35% with an increment of 5% in every succeeding batch of extracted chicken protein were studied in a separate experiment. The changing pattern of proteins within the samples when fiber was incorporated was studied by SDS- polyacrylamide gel electrophoresis. Detailed gel structure that may have caused the texture improvement or deterioration was studied using the Scanning Electron Microscopy (SEM). Specific characteristics of rhubarb fiber that may support the improvement were also studied using the light microscope.

## 5.2. Experimental

The areas covered in this section of the research were:

1. Effect of different fiber levels on the texture of chicken surimi and the moisture content of the product (texture and moisture analysis)

2. Dispersion of proteins within chicken surimi gel, when rhubarb fiber was incorporated (SDS- PAGE study)

3. The structure of the rhubarb fiber which may influence the texture modification of the chicken surimi gel (light microscope study)

4. Behavior of rhubarb fiber in chicken surimi gels (SEM studies).

**5.2.1. Effect of different fiber levels**

The products with different fiber levels were analyzed for the texture using the penetrometer which had been used for texture analysis in the texturization experiments. The moisture content of the samples were analyzed following the moisture analysis procedure by drying at 105°C for 18 h (24.003, AOAC, 1980)

Chicken protein samples were prepared following the same procedure as in the sample preparation for sensory evaluation, except for the percentage of fiber incorporated into each batch. Fiber was incorporated as 0%, 5%, 10%, 20%, 25%, 30%, 35% of the chicken protein extract.

**5.2.2. Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS- PAGE)**

Protein samples were analyzed by SDS- PAGE to study possible protein-protein interactions through the process and/or high concentration of any particular type of proteins in fiber rich areas of the samples. The samples analyzed by SDS- PAGE were: 1) raw MDCM, 2) MDCM after 5 washes with salt solutions, 3) after adding cryoprotectant mix to the extract 4) after freezing with cryoprotectant, 5) after gelling at 55°C with added 3% NaCl and fiber, and 6) after gelling at 55°C with added 3% NaCl. Protein extractions were made by dissolving 0.05 g sample in 10 mL 1% SDS solution. Gels were run through the scanning densitometer (Model - GS 300 Transmittance/Reflectance Scanning Densitometer, Hoefer Scientific Instruments, San Fransisco, USA) for qualitative analysis. Beef myofibril (obtained from biochemistry laboratory, Dept. Food Science, U of A, Edmonton, Alberta) was used as the standard sample in qualitative identification of the proteins. The method used by Laemmli (1970) was followed in the SDS- PAGE analysis.

Gels were set in a chamber in which 5 glass plates were clipped together with 0.75 mm spacers. The running gels were prepared by using 10.0%

acrylamide (SIGMA Chemical Company, St. Louis, MO) gels. The formula for running gel and stacking gel are given in Table 5.1. Ingredients for the running gel were mixed, excluding the ammonium persulfate ($(NH_4)_2S_2O_8$ - 98% powder, SIGMA Chemical Company, St. Louis, MO) and N,N,N',N'-tetramethyl-ethylenediamine (TEMED - 99% liquid, SIGMA Chemical Company, St.Louis, MO) The mixture was degassed for 5 min and then mixed with $(NH_4)_2S_2O_8$ and TEMED. Using a Pasteur pipette, the solution was filled through the sides of the chamber until the level reached the upper marking in the chamber. The spacer was inserted into each gel to remove air bubbles. Isobutanol (Fisher, Scientific, Edmonton, Alberta) was added into each gel 3 drops at a time (left, middle and right) until a layer of approximately 1 cm was formed above the gel. Gels were allowed to polymerize for 2 h. Isobutanol was subsequently washed off with distilled water. The upper surface of the polyacrylamide gel and the walls of the plates above the gel were dried. The stacking gel was filled into the space above the polyacrylamide running gel. Well-spacers were immersed in the upper gel. The upper gel was allowed to polymerize for at least 1 h. After polymerization the chamber was disassembled, the spacers were removed and the wells were rinsed with de-ionized water to remove any unpolymerized gel.

The gels were assembled in the electrophoresis chamber and inoculated with 5 μL protein sample in each of the wells. Electrophoresis was carried out until the tracking gel reached the bottom of the running gel (3-4 h). The gels were stained in a solution of 45% methanol, (Fisher Scientific, Edmonton, Alberta) 10% acetic acid (Fisher Scientific, Edmonton, Alberta) and 0.35% bromophenol blue "electran" (BDH Inc., Edmonton, Alberta) and then destained in 10% acetic acid (Fisher, Edmonton, Alberta) and 30% methanol solution (Fisher Scientific, Edmonton, Alberta). The amount of the protein of the corresponding band was

evaluated in the densitometer (Model - GS 300 Transmittance/Reflectance Scanning Densitometer, Hoefer Scientific Instruments, San Fransisco, USA).

### 5.2.3. Fiber structure study

It was necessary to study the structure of the fiber in order to understand the reasons for texture improvement when rhubarb fiber was incorporated into a chicken surimi sample. The lignified, cellulose helical coil structure is the organ used for transportation of water, nutrients and metabolites in the living plant. The behavior of the extracted fiber when mixed with protein samples was partially elucidated in scanning electron microscopy study. Two assumptions could be made regarding the roles of rhubarb fiber in the texturized chicken surimi: 1) the physical property of lignified fiber structure provides strength to meat gel due to its physical structure, thereby allowing the consumer to feel fibrous effect in the mouth, 2) the physiological characteristic of fiber modifies the gel structure within the sample thereby allowing the consumer to feel similar 'natural' meat texture in the mouth.

Rhubarb stalks were sliced with a scalper blade, crosswise and longitudinally, and slides were made to study the structure of rhubarb fiber. Fiber were also crushed into small particles using a mortar and pestle. Slides were made to study the structure of individual rhubarb fibers. Sections were fixed on the slides by gradual direct heating with a Bunsen's burner and photographs were made through the light microscope (Model KF2, Brightfield and phase contrast microscope, Carl Zeiss, West Germany).

### 5.2.4. Scanning electron microscope studies

The Scanning electron microscope (Cambridge S 250, UK) was used to observe the ultra structure features of samples from different stages of the process. Samples were prepared from the raw material stage to the final chicken protein gels

with and without fiber. Samples were made of 1) fiber alone, 2) fiber with salt soluble protein gels and 3) salt soluble protein gel alone. In the preparation of samples of fiber embedded in soluble protein-gel, the following procedure was utilized: Extracted meat proteins were vacuum packaged in water impermeable polyethylene bags. A bundle of rhubarb fiber was inserted in the same pouch prior to vacuum packaging. Sample bags were cooked at high temperature (90°C) in a water bath for 30 min. The fiber bundle was then allowed to suspend in the liquid phase which had separated from the fibrous part of the meat sample.

Samples were filled into cells in the sample hold with a pair of tweezers and frozen in nitrogen slush (-207°C, a mixture of frozen and liquid nitrogen), fractured with a pointer and sublimed at -40°C in the cryo-system (EMITEK cryo-system EMITEK, UK) for 30 min. Thereafter they were sputter coated with gold to a thickness of 200 μm. The structure of the gels and rhubarb fiber was observed through the SEM. Samples observed under the SEM were: 1) raw mechanically deboned chicken, 2) extracted meat protein mixed with cryoprotectant, 3) extracted meat protein samples mixed with cryoprotectant and rhubarb fiber, 4) meat proteins after gelling, 5) meat proteins after gelling with fiber, 6) fiber alone, 7) fiber with salt soluble proteins and 8) gel of salt soluble proteins alone. Selection of these particular samples for observation were aimed at studying the causes for texture improvement of meat protein gels when rhubarb fiber was incorporated.

## 5.3. Results and discussion

The results obtained by objective analysis of samples with different fiber levels (10-30%) are shown in Figure 5.1. The maximum force shown in the strip chart is described as the hardness. It shows that the hardness decreases with the increase of fiber level at a high rate up to 15%, thereafter the rate reduces. The sample with 25% fiber seems to have behaved unusually. The changing pattern of moisture

content of the product when different levels of fiber were incorporated is shown in Figure 5.2. High hydrophilic characteristic of polysaccharides in fiber may have increased water holding capacity of the product when fiber content of the product was increased. The capillary effect also may have supported this property of fiber coils. This was clearly observed in the fiber structure under the light microscope. The movement of the air bubble in the spiral coil was similar to the movement of an air bubble trapped in a capillary tube.

SDS-PAGE protein samples were compared with a standard sample of beef myofibrils. The bands of the samples were similar to that of beef myofibrils. They were scanned through a densitometer and the densitogram obtained is shown in Figure 5.3. The results obtained for qualitative (myosin, actin and tropomyosin) and quantitative (percentage) analysis are shown in Table 5.2.

Results obtained through the light microscope show (Figure 5.4) that a rhubarb fiber strand is a bundle of helical coils surrounded by a number of smaller vessels (Picture 'O'). The helical structure was not destroyed even after direct heat fixing (Picture 'P'). Although these tunnels were of spiral shape, they appear to have a solid wall through which liquid or air can pass. This was deduced after observing air bubbles moving through the spiral fiber coil when a drop of water was placed on the dry sample (Picture 'Q'). This movement of water through the fiber also gave evidence for fiber acting like a wick to liquid phase by allowing liquid to flow through the spiral coil.

Scanning electron micrographs of selected samples are shown in Figures 5.5. and 5.6. When comparing the pictures obtained for fiber alone (A,B,C,D) and fiber in clear gel- the gel made of salt soluble proteins (I and J), it can be seen that the clear gel forms a gel matrix within the rhubarb fiber coil. The samples of fiber bundles alone (C and D) showed a very sharp coil structure of the bundles. A number

of such coils were found to be arranged together, surrounded by smaller fibers (A and B). Chicken surimi gel which had no fiber incorporated had an even structure with a well defined gel matrix. Clear gel on its own (E,F,G,H) showed a very fine gel structure when compared to the surimi gel. When rhubarb fiber was embedded in clear gel (I and J), a larger gel matrix compared to that of surimi gel was observed. When rhubarb fiber was incorporated with chicken surimi samples, a structure similar to that of fiber in clear gel was observed inside the fiber coil (K,L,M,N) while outside the coil the structure was similar to that of the surimi gel structure on its own.

## 5.4. Conclusions

Increasing levels of fiber in the product helped to increase the moisture content of the end product. As can be seen in Figure 5.2, increasing level of fiber in the product has a linear relationship with the moisture content of the product.

When comparing the structures of samples observed through scanning electron microscopy, between fiber alone (C) and fiber in clear gel (I and J), it was revealed that salt soluble proteins formed a gel matrix which could be observed after subliming the liquid phase at increased temperature (-40°C in cryo system), whereas fiber alone showed a clear spiral coil. Clear gel alone showed a very fine gel structure (E,F,G,H) when only protein-protein interactions were possible. The structural difference when gel was formed within the fiber coil may have resulted from the polysaccharide-protein interaction, which may be a predominant feature in the structure formation of the product in comparison with the protein-protein interaction.

A similar gel structure to that of soluble proteins with fiber was formed within the fiber coils in surimi gel samples when fiber was incorporated. This suggested that salt soluble proteins were extracted and trapped by the fiber coil in the gelling process. However, the larger gel matrix within the fiber coil, which is

suspected to be formed by salt soluble proteins, may have created different texture properties within the surimi sample when fiber was incorporated.

As indicated in Table 5.2, high concentration of low molecular weight myofibril (myosin) was observed in surimi gels when fiber was incorporated into the chicken extract. The amounts of actin and tropomyosin were reduced. This reduction may have resulted from fiber acting like a wick in absorbing salt soluble proteins. Due to the small sample size used (50μg), an even distribution of proteins was not obtained. However, it could be assumed that the concentration of low molecular weight myofibrils were high when fiber fragments were included in the samples. These samples already have gone through the temperature-time treatment in gelling. According to Rodger (1992), myosin begins to denature at 37°C, actin after 65°C. It is possible that myosin content in the liquid phase was high at this stage. This may have resulted in high myosin levels where the fiber was plentiful.

When all the results are considered, it may be concluded that increased quantities of rhubarb fiber increase the moisture content of the product which in turn, increases the liquid phase within the surimi sample. Mixing the surimi samples with 3% NaCl increased the ionic strength of the media, allowing salt soluble proteins to extract into the liquid phase. During the heat treatment these soluble proteins solidify within the fiber structure forming a different gel matrix as compared to other areas of the sample. This may have caused unevenness of texture within the meat gel which was reported by the sensory panel as improved chewiness of the product.

## 5.5. References

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Chung, K.H. and C.M. Lee. 1990. Relationship between physicochemical properties of nonfish protein and textural properties of protein-incorporated surimi gel. J. Food Sci. 55:972-975, 988.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Rodger, G., 1992. The control and generation of texture in soft manufactured foods. In: Feeding and the texture of food (Vincent, J.F.V. and P.J. Lillford). pp. 211-227. Cambridge Uni. Press. New York.

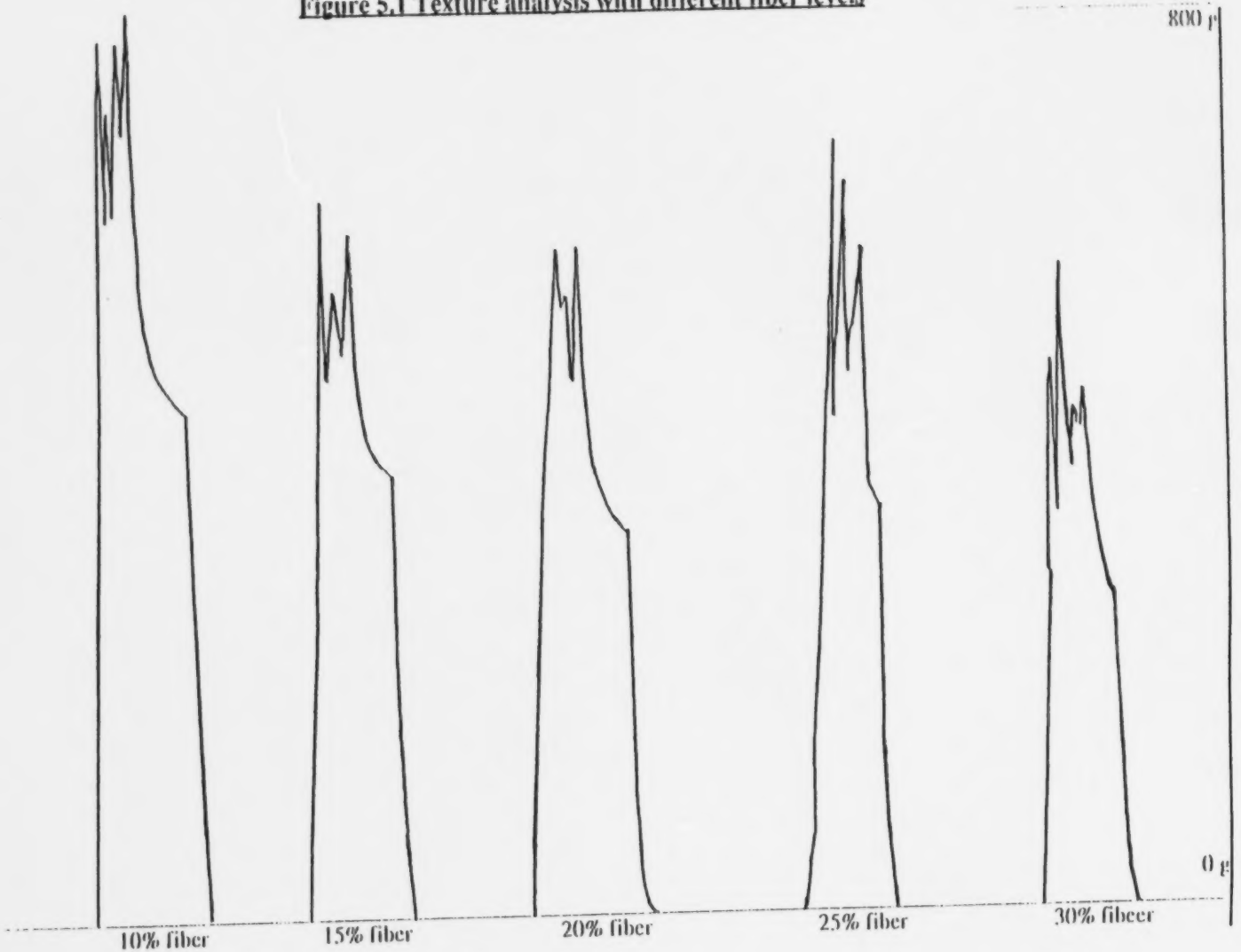
**Table 5.1 SDS-PAGE running and stacking gel formulas**

| | |
|---|---|
| Running gel (10%): | |
| Lower buffer stock*, (mL) | 7.50 |
| 30% acrylamide + 0.8% Bis;(mL) | 10.00 |
| Glycerol (60%), (mL) | 0.60 |
| Distilled water, (mL) | 11.80 |
| 3.3% $(NH_4)_2S_2O_8$, (μL) | 120.00 |
| TEMED, (μL) | 10.00 |
| Stacking gel: | |
| 30% acrylamide + 0.8% Bis.(37.5:1 ratio), (mL) | 1.34 |
| Upper buffer stock,** (mL) | 2.50 |
| Distilled water, (mL) | 6.00 |
| 10% $(NH_4)_2S_2O_8$, (μL) | 7.00 |
| Bromophenol blue in $H_2O$ (μL) | 10.00 |
| TEMED; (μL) | 10.00 |

* - Lower buffer - 1.5 M Tris -HCl, pH 8.8 plus 0.4% SDS

**- Upper buffer - 0.5 M Tris - HCl, pH 6.8 plus 0.4% SDS

**Figure 5.1 Texture analysis with different fiber levels**



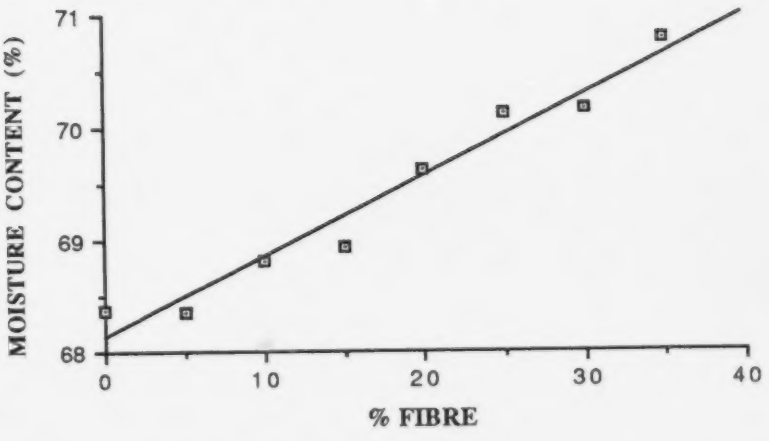



**Figure 5.2 Moisture in fiber-incorporated samples**

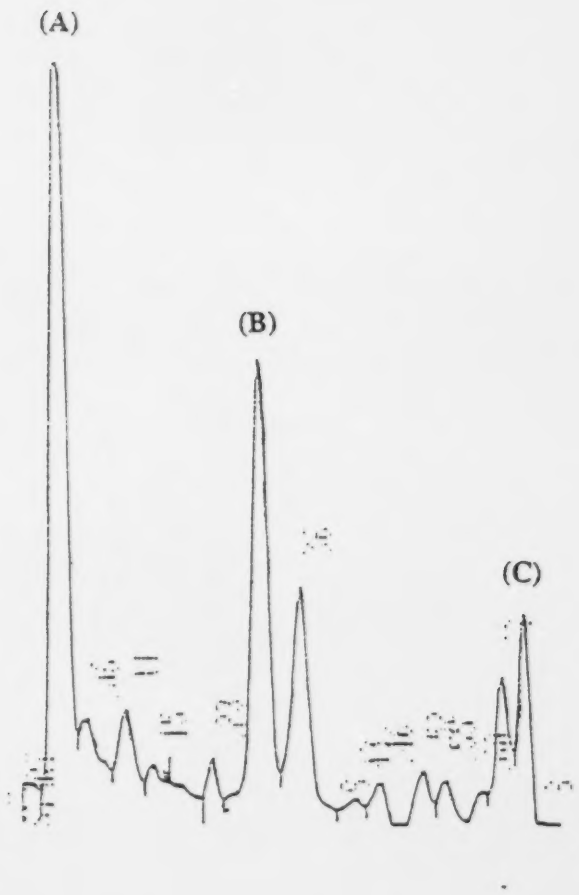



**Figure 5.3 Densitogram of the gel**

(A) - myosin; (B) - actin; (C) - tropomyosin

GS 300 transmittance/reflectance scanning densitometer
(Hoefer Scientific Instruments. San Francisco)

**Table 5.2 Types of proteins in meat extracts**

| Sample | Protein | | |
|---|---|---|---|
| | % Myosin | % Actin | % Tropomyosin |
| Beef MF | 36.67±0.45 | 23.68±0.29 | 14.82±0.90 |
| Raw MDC | 25.48±2.43 | 24.44±2.11 | 16.01±1.27 |
| W-MDC+C | 39.82±0.29 | 17.63±0.59 | 10.15±0.45 |
| W-MDC+C+F | 39.48±0.14 | 21.60±0.18 | 11.32±0.05 |
| W-MDC+C+F+S | 48.31±0.27 | 15.36±0.11 | 9.12±0.13 |
| W-MDC+C+S | 39.48±3.14 | 20.11±1.00 | 11.72±1.03 |

Beef MF - Beef MF- Beef myofibrils

MDC - Mechanically deboned chicken

W-MDC - Washed mechanically deboned chicken

C - Cryoprotectant

F - Rhubarb fiber

S - Salt

**Figure 5.4 Photographs of rhubarb fiber.**

**O** - Photograph of rhubarb fiber (cross section)

**P** - Light microscopic photograph of extended rhubarb fiber coil

**Q** - Light microscopic photograph of rhubarb fiber with an air buble trapped inside

O


P


Q

**Figure 5.5 Scanning electron micrograph of rhubarb fiber and meat gels**

**A, B, C, D -** Microphotographs of rhubarb fiber.

**E, F** - Microphotographs of clear gel (salt soluble proteins)

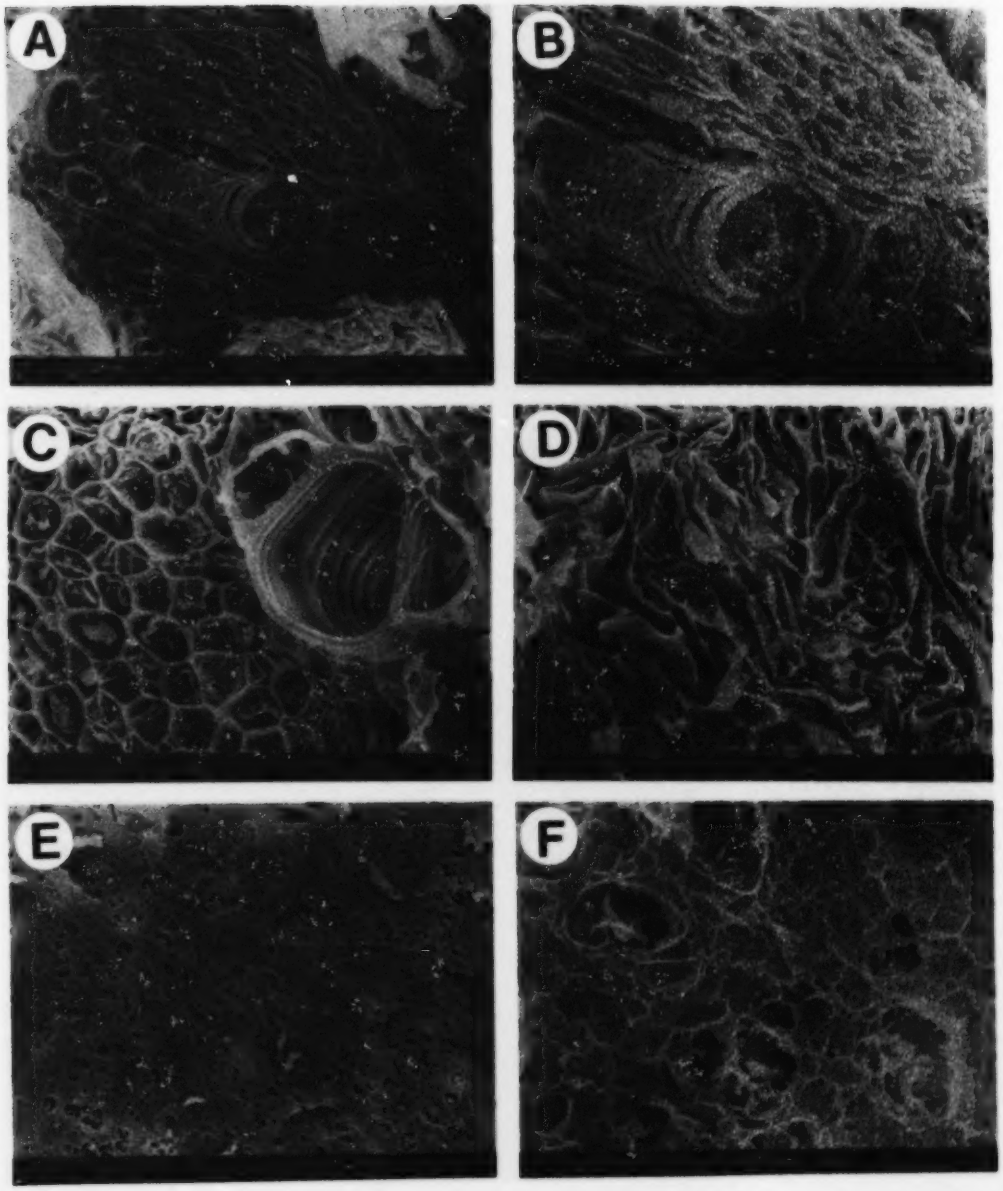
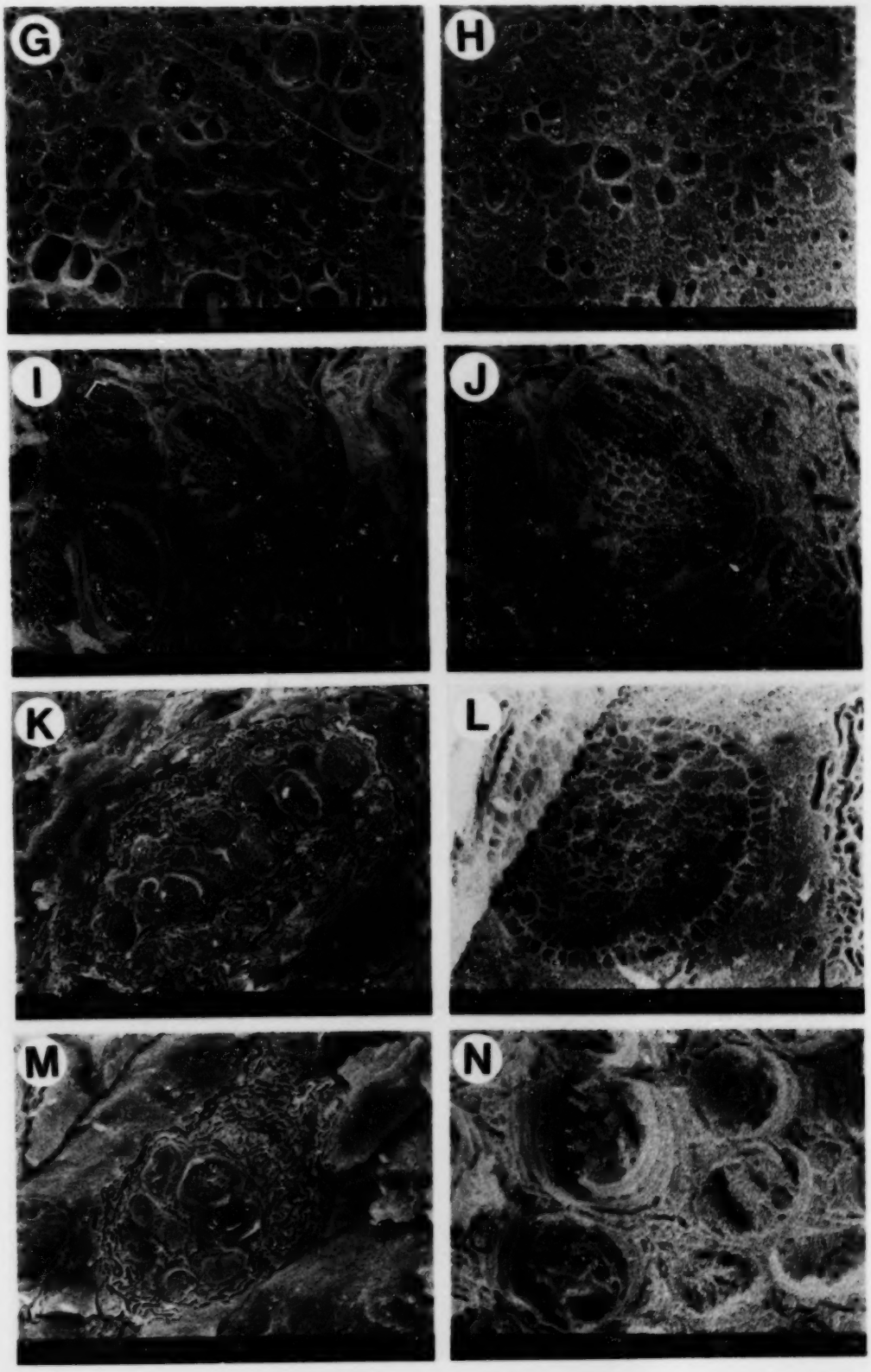

A
B
C
D
E
F

**Figure 5.6 Scanning electro micrograph of meat gels**

**G, H** - Microphotograph of the clear gel (salt soluble proteins)

**I, J** - Rhubarb fiber in clear gel

**K, L, M, N** - Rhubarb fiber in chicken surimi gel.


G
H
I
J
K
L
M
N

# Chapter 6. Process and Product Safety

## 6.1. Introduction

Product and process safety was analyzed at Critical Control Points. Samples were analyzed for total bacterial counts (TPC) and *Salmonella* where necessary. Storage tests were carried out with the end products in order to determine both the safe storage conditions and the appropriate length of storage. Thiobarbituric acid analysis (TBA) was carried out to determine the degree of rancidity of the product during storage. Microbiological analysis (TPC) was also carried out on these samples to ensure the wholesomeness of the product.

## 6.2. Hazard analysis at critical control points

Hazard analysis at critical control points (HACCP) is expected to indicate any enrichment or dilution of bacterial counts, contamination with foreign matter induced by materials and/or machinery used, biochemical degradation of the product, etc., at various critical stages of the process. This is a modern term to get at the Cause and Effect problems. A process hazard is a possible source of trouble along the processing line which might be defined as a failure to identify 1) critical material, 2) critical processing points, 3) adverse environmental conditions, and 4) human malpractice (Gould and Gould, 1988). These analyses would indicate the presence and/or introduction of any component to food product that could initiate and/or cause health hazards due to inadequate control at critical points in a process. Critical control points (CCP) are commonly identified as the stages where parameters such as temperature, time, addition of new ingredients, mixing, human handling etc. are involved in a process. Poultry products are, in general, considered to be high in bacterial counts. Contamination from the slaughtering environment is largely responsible for the increased number of microorganisms, especially Coliforms,

*Escherichia coli*, Enterococci, *Staphylococcus aureus*, *Clostridium perfingens* and *Salmonella*. In addition, the raw material used in this research project, i.e. mechanically deboned chicken meat (MDCM), can be further contaminated with bacteria introduced by man and equipment during processing. *Salmonella* is recognized as the main pathogen associated with poultry. Reduction of this organism in poultry is a major challenge facing the food industry (Todd, 1980). Furthermore, in preparation of chicken surimi there are a number of factors, (critical points) e.g. raw material, holding time in salt solutions during washing, temperature-time treatment, addition of rhubarb fiber etc. which could greatly affect the enrichment of microorganisms already present in the raw material. As reported by Bryan (1980), some of the processes similar to these have resulted in food born diseases from 1968 to 1977. According to Bryan (1980) the most frequently identified factors that resulted in these outbreaks were: cooling of cooked foods (48%), food prepared a day or more before serving (34%), inadequate cooking or thermal processing (27%). Commonly reported food-borne diseases associated with these problems were staphylococcal intoxication, salmonellosis, *C. perfringens* gastroenteritis, and trichinosis. In this project, considering the hazardous factors involved in the process, microbial analysis was carried out at the critical points. The purpose of carrying out these analyses was to eliminate or minimize possible microbial hazards prior to recommending the proposed process for a scale-up project which eventually would produce a consumer product. Due to paucity of information currently available on this particular product regarding the survival or multiplication of any microorganisms through the process, it was also necessary to analyze the products prior to presenting it to a sensory panel.

### 6.2.1. Critical points

Critically hazardous points considered for microbial analysis in the process were: raw materials, washing with salt solutions, mixing with salt and rhubarb fiber, temperature time treatment (gelling), and product presented to the taste panel (deep fried product). Microbiological analyses were also carried out with chicken nuggets which were used as the standard in sensory evaluation. At each critical point, products were analyzed for total plate count and *Salmonella*. Analysis was carried out in duplicate for four batches of meat gels processed for one sensory evaluation session (two batches with fiber and two batches without fiber).

## 6.3. Procedure for Total Plate Count (TPC)

In analyzing TPC at CCP, the spread plate method was used (Busta et al., 1984). The extent of bacterial contamination of MDCM samples through various processing steps was examined by surface plating at appropriate dilutions. Samples of 25 g each were diluted in 225 mL of 0.1% peptone water (Difco Laboratories, Detroit, Michigan, USA). Ten fold serial dilution was prepared and 0.1 mL volume was surface plated onto Tripticase Soy Agar (TSA, Difco Laboratories, Detroit, Michigan, USA). Plates were incubated at 35°C for 48 h before enumeration.

## 6.4. *Salmonella* analysis

Members of the genus *Salmonella* are infectious pathogens capable of initiating clinical symptoms in humans. A goal for food processing plants is to prevent *Salmonella* contamination of food products, which depends to a great extent upon an adequate quality control program (Papa et al. 1991). Microbiological analysis at critical points at the research and development stage of food processing enables the researcher to develop measures for an assurance program in scale up processes. In this research project samples were analyzed for *Salmonella* at the critical points already

described. *Salmonella* was isolated according to the HPB (Health Protection Branch, 1983) methodology.

**6.4.1. Procedure**

Twenty five (25) g of samples were pre-enriched in 250 mL lactose broth (Difco Laboratories, Detroit, Michigan, USA) for 24 h at 35°C. *Salmonella typhimurium* (ATCC.13311, obtained from microbiology laboratory, Dept. Food Science, U of A, Edmonton, Alberta) was used as a positive control culture. Selective enrichment of the cultures were made into Selenite Cysteine (SC - Difco Laboratories, Detroit, Michigan, USA) and Tetrathionate Brilliant Green broth (TBG - Difco Laboratories, Detroit, Michigan, USA), following incubation for 24 h at 35°C for SC broth and 43°C for TBG broth. Cultures were streaked onto Bismuth Sulfite (BS - Difco Laboratories, Detroit, Michigan, USA) and Xylose Lysine Desoxycholate (XLD - Difco Laboratories, Detroit, Michigan, USA) agars. XLD plates were incubated at 35°C for 24 h. BS plates were examined after both 24 and 48 h of incubation at 37°C. Typical colonies from both XLD and BS agars were identified according to their appearance on the selective plating media . On XLD plates, pink colonies with or without black centers and on BS agar plates, *Salmonella* appeared as brown or black with or without a metallic sheen.

**6.4.2. Purification**

Suspected colonies were streaked onto Tripticase Soy Agar (Difco Laboratories, Detroit, Michigan, USA) for purification. Plates were incubated for 18 h at 35°C.

**6.4.3. Biochemical screening**

Pure cultures of the isolates were grown in Trypticase Soy Broth (TSB - Difco Laboratories, Detroit, Michigan, USA) for biochemical screening. Inoculated media was incubated at 35°C for 18-24 h. Triple sugar iron (TSI - Difco Laboratories, Detroit, Michigan, USA) agar slants were inoculated with typical suspicious colonies. Center portion of colonies was picked with a needle and the TSI slant were streaked. The slant were then stabbed up to the bottom of the test tube with the same needle. Without flaming Lysine Iron (LI - Difco Laboratories, Detroit, Michigan, USA) agar slants were inoculated in the same manner. TSI agar slants were incubated for 24 h at 35°C. LI agar slants were incubated at 35°C and examined after 24 h and then continued incubation for further 24 h before examining.

Additional biochemical screening tests included the production of urease and oxidase. A small amount of growth from the presumptive positive TSI agar was transferred to urea broth (Difco Laboratories, Detroit, Michigan, USA) with a sterile needle. An uninoculated tube was included as a negative control. Tubes were incubated for 24 h at 35°C. *Salmonella* growth was confirmed by a negative reaction where the broth did not turn purple red color. In oxidase test, oxidase reagent was applied on a filter paper. Colony samples from TSI agar (Difco Laboratories, Detroit, Michigan, USA) was tested on this filter paper. The change of color of the filter paper indicated the possibility of *Salmonella* contamination in the tested samples. Suspicious colonies were further analyzed by potassium cyanide test. Tryptophane broth (Difco Laboratories, Detroit, Michigan, USA) was inoculated with a small amount of growth from TSI agar culture, incubated for 24 h at 35°C, and then transferred a loop full (3 mm) of *Salmonella* enriched tryptophane broth culture to KCN broth (KCN, SIGMA, St.Louis, MO). Tubes were sealed with wax coated

stoppers and incubated for 48 h at 35°C. Tubes were examined for turbidity as a positive reaction for *Salmonella* growth.

### 6.5. Storage tests

At this stage the most suitable form of the product for commercialization has not been determined. Depending upon the target market segment and the potential application of the extracted meat batters, the product could reach the immediate customer either in cooked form or in raw batter form. Hence storage tests were carried out with both the gelled (heat treated) and ungelled chicken proteins. Samples were tested under two storage temperatures, i.e. stored at 4°C and -30°C. Microbial and chemical analyses were carried out as measures of the keeping quality of the products.

#### 6.5.1. Microbial analysis

The samples analyzed for microbial quality (Busta et al., 1984) were extracted chicken proteins with and without rhubarb fiber, and extracted chicken proteins after gelling, also with and without fiber. Fiber alone was analyzed for initial bacterial count to ensure it was not a source of contamination. Samples were weighed in 5 g lots and stored at 4°C and -30°C. Dilution of the samples was made with 45 mL 0.1% peptone water. Further dilution was made with 1 mL of the preceding dilution in 9 mL 0.1% peptone water. Pour plate method was used with Tripticase soy agar (TSA-Difco Laboratories, Detroit, Michigan, USA). Plates were incubated at 37 °C for 48 h and examined for plate count.

#### 6.5.2. Chemical analysis

Lipid oxidation of meat products is one of the major concerns in deterioration of flavor, color, texture and nutrients of the products. The most common

measurement of lipid oxidation in muscle foods is the determination of the 2-Thiobarbituric acid (TBA) number, defined as the mg malonaldehyde/kg of meat which is commonly called the TBA-test (Melton, 1983).

The TBA number was measured by the extraction procedure of Salih et al. (1987). Four replications were made for each treatment. Eighteen mL of 3.86% perchloric acid (BDH, Inc., Toronto, Ont.) and 50 mL butylated hydroxy anisol (BHT) solution (SIGMA, St. Louis, MO.) were placed in test tubes. Two grams of samples were weighed into these test tubes and homogenized with a Brikman Polytron (type PT 10/35, Westbury, NY) for 15 seconds at high speed (set at 7). The homogenate was filtered through a Whatman #1 filter paper. Two mL of the filtrate were mixed with 2 mL of 20 mM TBA (SIGMA, St. Louis, MO.) in distilled water and incubated at room temperature in the dark for 15 h. Concentration of a complex compound that malonaldehyde formed with TBA was determined by measuring its absorption at 531 nm on a spectrophotometer against a blank containing 2 mL distilled water and 2 mL of 20 mM TBA solution. TBA numbers were expressed as milligrams malonaldehyde per kg chicken protein sample.

## 6.6. Results and Discussion

The results of total microbial counts-analyses are shown in Table 6.1. Standard plate counts for all four uncooked batches averaged $3.5 \times 10^4$ cfu/g. Washed samples gave lower plate counts, while samples mixed with fiber, salt and cryoprotectant resulted in slightly higher counts compared to the washed ones. No detectable count was observed after gelling at 55°C for 4 h or in deep fried samples. Commercial nuggets were low in counts in either uncooked or cooked samples.

*Salmonella* was identified using the API 20E system (1986). Following the API number 6704752 - *Salmonella* excellent identification 1 in 12

chances - presence of *Salmonella* in uncooked samples was confirmed. Results obtained from analysis of *Salmonella* for all four batches of samples at each of the critical points are reported in Tables 6.2 and 6.3. Results of bacterial counts during the 30 d storage of cooked and uncooked samples are shown in Table 6.4.

From the results obtained for bacterial counts at critical control points, it was observed that washing reduced the total bacterial count. Addition of salt, fiber and cryoprotectant and further chopping increased the count slightly, but not to a level as high as in the raw samples. Gelling at 55°C for 4 h reduced the micro-organisms to undetectable levels. As the product was handled for approximately 3 h during the cooking preparation, numerous chances existed for cross contamination to occur. Proliferation of the microorganisms may also occur during this period. However, deep frying of the samples before serving to the taste panel ensured wholesomeness of the product. Results from these analyses showed that washing reduced the number of microorganisms on the samples considerably as compared with the raw samples.

The results obtained for TPC during the storage are given in Table 6.4. During the storage, samples stored under frozen conditions, both cooked and uncooked, had acceptable levels of counts even after 21 day storage period (usually when the counts are higher than $10^7$, the products are unacceptable). In the cold storage, the uncooked samples had high bacterial counts on the 21-day analysis. According to the TPC this could be inferred as unacceptable after 14 days if stored under cold conditions.

The TBA numbers obtained during storage of uncooked samples with and without fiber, stored under cold (4°C) and frozen (-30°C) conditions are given in Table 6.5. A TBA value higher than 2 has been reported (Melton, 1986) to give rancid flavor to a product during the storage. Our results show that none of the

samples developed this level of TBA number. The results for TBA analyses for cooked samples with fiber are given in Table 6.6. These samples also yielded low TBA numbers after 17 day storage.

## 6.7. Conclusion

The results indicated that although *Salmonella* was not detected in the raw MDCM, it could multiply to detectable level during processing, especially during washing and addition of other ingredients. Gelling at 55°C for 4 h killed any *Salmonella* which may have been present in the uncooked samples. Wholesomeness of samples presented to the taste panel was further assured by deep frying. These results indicated that a product processed with the procedure used in this research project could reach the consumer *Salmonella* free. Given the undetectable levels of TPC in samples cooked at 55°C for 4 h and in the deep fried sample, it can be concluded that a product processed under conditions suggested here is microbiologically safe.

In the frozen storage, samples with fiber showed a significant difference in TBA values as compared with the samples with no fiber after 2 d, 16 d, and 30 d stage periods. Cooked samples did not show any difference in TBA values during the storage.

Deterioration of products of this nature may be caused mainly by microorganisms rather than rancidity if stored under cold conditions. The increase in total plate counts to an unacceptable level during the refrigeration storage indicates the unsuitability of the refrigerated storage for this type of products. Identification of microflora during the storage was not carried out. Product suitability for further storage mainly depends on the type of microflora present in the samples. Therefore, it is not obvious whether these high levels of microbial counts indicate a product

deterioration. However, at this level of microbiological study, it can recommended that a frozen storage would be more suitable for storage of this product.

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**Table 6.1 Total plate count at critical control points**

| Critical Points | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) | | | (2) | | | (3) | | | (4) | | | (5) | | | |
| sample | count | | sample | count | | sample | count | | sample | count | | sample | count | | |
| R1 | 3.5 | x $10^3$ | W1 | - | | WS1 | 1.1 | x $10^3$ | G1 | 0 | | C1 | 0 | | |
| R2 | 3.3 | x $10^3$ | W2 | - | | WS2 | 1.3 | x $10^3$ | G2 | 1.5 | x $10^1$ | C2 | 0 | | |
| R3 | 3.8 | x $10^3$ | W3 | 1.5 | x $10^3$ | WS3 | 1.2 | x $10^3$ | G3 | 0 | | C3 | 0 | | |
| R4 | 3.7 | x $10^3$ | W4-1 | 6.3 | x $10^2$ | WS4 | 1.5 | x $10^3$ | G4 | 3.5 | x $10^1$ | C4 | 6.5 | x $10^1$ | |
| NR1 | 6.5 | x $10^2$ | | | | | | | | | | NC1 | 6.0 | x $10^1$ | |
| NR2 | 5.0 | x $10^2$ | | | | | | | | | | NC2 | 3 | x $10^1$ | |

(1) Raw material stage
(2) After washing
(3) Salt added after washing
(4) Gelled at 55°C for 4h
(5) Deep fried

R1, R2, R3, R4- Raw samples
W1, W2, W3, W4- Samples after four washinfg cycles
WS1, WS2, WS3, WS4- Samples after adding cryoprotetcant, salt and fiber
G1, G2, G3, G4- Samples after gelling at 55°C for 4 hours
C1, C2, C3, C4- Samples deep fried for tasting
NR and NC- Commercial chicken nuggets Raw and Cooked

**Table 6.2 *Salmonella* screening results**

| Sample | (XLD) TTBG | (XLD) SCB | (BSA) TTBG | (BSA) SCB | Salmonella |
|---|---|---|---|---|---|
| R1 | Atyp | Atyp | Atyp | Atyp | - |
| R2 | ND | ND | ND | ND | - |
| R3 | ND | ND | ND | ND | - |
| R4 | ND | ND | ND | ND | - |
| NR1 | NG | NG | NG | Atyp | - |
| NR2 | Atyp | Atyp | Atyp | Typ | - |
| W1 | Typ | O/G | O/G | O/G | + |
| W2 | Typ | Atyp | O/G | O/G | + |
| W3 | Atyp | Atyp | O/G | O/G | + |
| W4 | Typ | Atyp | O/G | O/G | + |
| WS1 | Typ | Atyp | Typ | Typ | + |
| WS2 | Typ | Atyp | Typ | Typ | + |
| WS3 | Atyp | Atyp | Typ | Typ | + |
| WS4 | Atyp | Atyp | Typ | Typ | + |
| G1 | Atyp | Atyp | Atyp | Atyp | - |
| G2 | Atyp | Atyp | Atyp | Atyp | - |
| G3 | ND | ND | ND | ND | - |
| G4 | ND | ND | ND | ND | - |
| C1 | NG | NG | NG | NG | - |
| C2 | NG | NG | NG | NG | - |
| C3 | NG | NG | NG | NG | - |
| C4 | NG | NG | NG | NG | - |
| NC1 | NG | NG | NG | NG | - |
| NC2 | NG | NG | NG | NG | - |

R1,R2,R3,R4- Raw meat samples
NR1, NR2- Uncooked chicken nugget samples
W1, W2, W3, W4- Samples after washing process
WS1, WS2, WS3, WS4- Washed samples after adding other ingredients
G1, G2, G3, G4- Samples after gelling
C1, C2, C3, C4- Deep fried samples
NC1, NC2- Cooked chicken nugget samples
Atyp- unusual
ND- Not Detected
Typ- presumptive *Salmonella*
NG- Not grown
O/G - over grown

**Table 6.3 *Salmonella*, biochemical screening results**

| sample | TSI | LIA | Urease | Oxidase |
|---|---|---|---|---|
| W1-1 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| W1-2 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| W2-1 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| W2-2 | ND | ND | ND | - |
| W3-1 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| W3-2 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| W4-1 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| W4-2 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| WS1-1 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| WS1-2 | K/A g | K/A g | + | - |
| WS2-1 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| WS2-2 | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| WS3-1 | A/A g | K/K | - | - |
| WS3-2 | A/A g | K/K | - | - |
| WS4-1 | K/A $H_2S$ | K/A | + | - |
| WS4-2 | K/A $H_2S$ | K/K $H_2S$ | - | - |
| NR1-1 | A/A g | K/A g | - | - |
| NR1-2 | A/A g | K/A g | - | - |
| St | K/A $H_2S$ g | K/K $H_2S$ | - | - |
| E Coli-1 | K/A | K/K | - | - |
| E Coli-2 | K/A g | K/K | - | - |

K/A - acid alkaline
K/K - acid acid
ND - not done
H2S g - hydregeon sulphide gas production
g - gas production
Urease (-) - *Salmonella* positive
Urease (+) - *Salmonella* negative
Oxidase (-) - *Salmonella* positive

**Table 6.4 Bacterial counts during the storage**

| Storage period | Cold storage | | Frozen Storage | |
|---|---|---|---|---|
| | Cooked | Uncooked | Cooked | Uncooked |
| '0' day | $0.0 \times 10^1$ | $1.5 \times 10^4$ | $0.0 \times 10^1$ | $2.2 \times 10^4$ |
| '7' day | $4.5 \times 10^1$ | $1.24 \times 10^6$ | $2.5 \times 10^1$ | $0.0 \times 10^3$ |
| '14' day | $1.2 \times 10^1$ | $7.5 \times 10^6$ | $1.5 \times 10^2$ | $1.6 \times 10^4$ |
| '21' day | $3.0 \times 10^1$ | $1.26 \times 10^9$ | $3.5 \times 10^2$ | $3.4 \times 10^4$ |

**Table 6.5 TBA number of uncooked samples during storage**

| Storage (day) | Frozen | | | Cold | | |
|---|---|---|---|---|---|---|
| | Without fiber | With fiber | SEM | Without fiber | With fiber | SEM |
| 0 | - | - | - | 0.74 | 0.69 | 0.022 |
| 2 | 0.46[a] | 0.62[b] | 0.008 | 0.85 | 0.99 | 0.056 |
| 9 | 0.47 | 0.63 | 0.035 | 0.92 | 0.92 | 0.112 |
| 16 | 0.58[a] | 0.40[b] | 0.021 | 0.60 | 0.66 | 0.058 |
| 23 | 0.42 | 0.55 | 0.076 | 0.84 | 1.00 | 0.085 |
| 30 | 0.32[a] | 0.56[b] | 0.048 | 0.62 | 0.64 | 0.030 |

SEM - standard error of the mean

* a and b in the same row are significantly different ($p = 0.05$)

**Table 6.6 TBA numbers of cooked samples, during storage**

| Storage condition | Storage (day) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 3 | 10 | 17 |
| Frozen | - | - | 0.45 | 0.30 | 0.32 |
| Cold | 0.68 | 0.54 | 0.42 | 0.44 | 0.31 |
| SEM | | | 0.097 | 0.032 | 0.093 |

SEM - standard error of the mean

## Chapter 7. General Conclusion and Recommendations

The work carried out in this research project reveals that mechanically deboned chicken meat (MDCM) can be used as a raw material in chicken surimi production. In this study, chicken protein extraction and gelling experiments were carried out in three stages. The aim was to obtain a standard procedure for producing a protein extract to be used as a base material for texturization processes involving the incorporation of rhubarb fiber. In the initial stage, processing variables which may have significant effects on the texture of chicken surimi gel were identified. This led to the second stage of experiments in which attempts were made to optimize the processing variables identified, based on the protein yield, the color, and the texture of the extracted product. The results revealed that suitable criteria for optimization were chemical composition and the color of the extract, but not the texture. Therefore, the standardized procedure for MDCM protein extraction chosen for use in the subsequent phases of the research consisted of 5 washing cycles of 7 min each, with 0.5% $NaHCO_3$ and 0.06% NaCl solution in the first and the second washes, respectively, followed by 3 washes, with 0.3% NaCl solution. The cryoprotectant which consisted of sucrose, sorbitol and sodium polyphosphates was added to the extracted protein (chicken surimi) at the rate of 11.4%, based on the weight of the protein and fiber, to improve the freeze-thaw properties of the gel during frozen storage. In the third stage of experiments, processing variables in the gelling process were optimized based on the texture characteristics of the gel. The optimum process chosen consisted of chopping the extracted protein with 3% NaCl in a silent cutter for 10 min and then heating at 55°C for 4 h.

In the process of texturization with rhubarb fiber, the physicochemical properties of fiber strands in relation to texture formation in the texturized product were evaluated under light and electron microscopes. The results revealed that salt

extractable protein formed a gel matrix within the hollow helical coils of fiber, while the rest of the protein matrix was formed outside the fiber strands. The fiber, being a complex carbohydrate and hydrophilic in nature, increased juiciness of the texturized product.

The minimum amount of rhubarb fiber incorporated into chicken surimi that showed a modest effect ($p=0.08$) on the texture of the product was 15%. However, the sensory panel showed preference toward a softer product. Therefore, the amount of the fiber incorporated was raised to 20%. The proportion of chicken surimi and rhubarb fiber may be adjusted, if there are changes in the process and/or ingredients, so that the final moisture of the texturized product is about 70%.

The improvement of product texture with the incorporation of rhubarb fiber appears to be due to three factors. Firstly, the fiber lends juiciness to the product by increasing its water holding capacity. Secondly, the fiber strands provide hollow tubes of helical fiber coils for salt-extractable protein to form a soft matrix within, while the rest of the protein forms a firmer structure around the fiber. Finally, the fiber strands themselves, which consist essentially of lignified cellulose coated with pectic substances, provide chewability to the otherwise pasty product. A combination of these three factors seems to provide a more natural mouthfeel to the product as compared to a commercial product made from ground chicken meat.

In addition to the improved mouthfeel, incorporation of fiber appears to impart a number of other desirable properties to the product. Samples which had fiber incorporated, seem to thaw faster compared to the ones without fiber. In freezing, fiber may have elevated the glass temperature of the product so that the texture of the product was hardly damaged by ice crystal formation. When frozen chicken surimi was thawed, it was a very sticky product which was difficult to handle manually. When fiber was incorporated this stickiness was reduced, thereby making

the molding or forming of the product into desired shapes easier when compared with product without fiber incorporated.

This research has shown that it is possible to use long strand vegetable fibers to improve textural characteristics of restructured surimi-type products. The fiber not only improves mouthfeel but also elevates nutritional value of the products due to the increase in the dietary fiber from plant sources. However, much work remains to be done. Further work should include the following:

1. Economic evaluation of the product and process.

2. Experiments with various sources of vegetable fiber and surimi.

3. More suitable methods for incorporation of fiber into surimi to obtain uniform distribution.

4. Product development based on this structured material and use of other ingredients to further improve the products.

5. Consumer and market testing of the products developed.

6. Technology transfer.